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Managing Fusarium Wilt in Watermelon Production

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MANAGING FUSARIUM WILT IN WATERMELON PRODUCTION

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ABSTRACT

Fusarium wilt of watermelon, caused by *Fusarium oxysporum* f. sp. *niveum*, was first described in 1894 in South Carolina and Georgia and has become a limiting factor in watermelon production worldwide. In recent years, restriction on use of the soil fumigant methyl bromide and the recent development of more virulent races of *Fusarium oxysporum* f. sp. *niveum* has created a critical need for alternative management techniques. In 2011-2013 field experiments were designed to evaluate the efficacy of incorporating a cover crop of hairy vetch into the soil to manage Fusarium wilt. Colony-forming units (CFU) of *Fusarium oxysporum* were sampled to evaluate differences among treatments as well as changes within a treatment over time. The incorporation of hairy vetch significantly increased CFUs of *F. oxysporum* from the baseline January sampling to the April sampling, post-incorporation of cover crops. The fumigants dimethyl disulfide + chloropicrin and methyl bromide + chloropicrin significantly reduced CFUs from the baseline sampling. Weekly disease ratings, percentage area covered by vines, and harvest data were collected and analyzed to see if the incorporation of hairy vetch reduced Fusarium wilt. In both 2012 and 2013, neither hairy vetch nor dimethyl disulfide + chloropicrin reduced disease incidence or increased yield when compared to the rye control. Separate field experiments were designed to compare the efficacy of grafting, soil fumigation, and a race 1-resistant cultivar on Fusarium wilt management. 'Tri-X 313', a susceptible cultivar, was grafted onto Emphasis (*Lagenaria siceraria*) and Strong Tosa (*C. maxima* X *C. moschata*) rootstocks. 'Fascination' was used as the race 1-resistant cultivar, and dimethyl disulfide + chloropicrin was chosen as

the fumigant. Disease incidence was assessed as in the cover crop study. The fumigant significantly lowered CFUs when compared to the rye control. In 2013, both rootstocks as well as the fumigant significantly reduced disease incidence when compared to the non-and self-grafted controls. 'Fascination' had a consistent disease rating of 15-20% in both years and had statistically lower disease than the non-grafted control in 2013. There were no differences in yield in any year among any treatments. It can be concluded from this two-year study that grafting was the best technique for Fusarium wilt management.

DEDICATION

To everyone who had a hand in making all of this possible.

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CHAPTER ONE

LITERATURE REVIEW

Introduction

Watermelon (*Citrullus lanatus* (Thunb.)) is an important fruit grown throughout the world with over 80,000,000 metric tons grown worldwide in 2002 (Egel & Martyn, 2007). In 2012, the United States grew 139,500 acres of watermelon which had a fresh market value of over 520 million dollars (Anonymous 2012b). Not only is watermelon important in the United States, it is also very important to the South Carolina economy. It is estimated that 8,000 acres of watermelon were grown in South Carolina in 2012, making it the sixth leading watermelon producing state. Two hundred and eighty-five million pounds of fresh market watermelons were produced in South Carolina which had a value of over 31 million dollars. Watermelon production promotes national, state, and local economies. Not only does watermelon production stimulate the economy, but it also has many health benefits for the consumer. Watermelons have high levels of vitamins A, B6, and C, which boost the immune system, cleave free radicals, and support eye and nerve health. Watermelon also contains potassium, which helps with cramping on hot summer days, and more lycopene than any other fresh fruit on the market (Anonymous 2013).

There are many diseases that affect watermelon, among them Fusarium wilt. Fusarium wilt is a major limiting factor for watermelon production around the world (Zhou & Everts, 2004; Zhou & Everts, 2007b; Zhou *et al.*, 2010). In the United States,

Fusarium wilt symptoms on watermelons are caused by the pathogen *Fusarium oxysporum* f. sp. *niveum* (FON) races 0, 1, 2, or 3. Each of these races can cause Fusarium wilt in watermelon, with race 3 being the most highly aggressive race (Zhou *et al.*, 2010). Fusarium wilt symptoms can be seen when a dull green foliar chlorosis appears on the foliage which is followed by wilting of the watermelon plant. A trademark of Fusarium wilt is dark, interveinal markings inside the vascular bundle when a runner is cut open (Kleczewski & Egel, 2011). In watermelon-producing regions of the eastern US, crop rotations upwards of 5 to 7 years may be required to control FON (Zhou & Everts, 2004). Because growers in these regions do not have sufficient land to follow rotation recommendations, selection for FON races has occurred (Keinath *et al.*, 2010) (Zhou & Everts, 2004). In recent years, surveys have revealed that the highly aggressive race 2 has become more prevalent in many watermelon-producing areas; six out of the top ten watermelon producing states have confirmed the presence of race 2 (Zhou & Everts, 2004; Zhou *et al.*, 2010). Currently, there are no watermelon cultivars that have any resistance to races 2 or 3 (Keinath *et al.*, 2010). For years, methyl bromide was used as a standard for controlling pests in soil environments in the production of fruits and vegetables. Recently, methyl bromide was banned as a soil fumigant due to depletion of the ozone layer, environmental risks, and human health hazards (Cohen *et al.*, 2007). With restriction on use of methyl bromide, populations of soil microbes are expected to shift with fewer chemical management techniques available (Cohen *et al.*, 2007; Rothrock *et al.*, 1995). With no resistant cultivars for races 2 or 3, very few cultivars with race 1 resistance, combined with increasing levels of soil pathogen inoculum, and

the phasing out of methyl bromide, FON-induced losses of watermelon production are expected to increase (Zhou & Everts, 2004; Zhou *et al.*, 2010).

Fusarium oxysporum Schlechtend.:Fr. f. sp. *niveum*

Fusarium oxysporum Schlechtend.:Fr. f. sp. *niveum* (FON) is the causal agent of the disease Fusarium wilt seen on watermelon. The pathogen is in the Kingdom Fungi; Division Ascomycota; Class Sordariomycetes; Order Hypocreales; Family Nectriaceae; Genus *Fusarium*; Species *Fusarium oxysporum* (Kleczewski & Egel, 2011). Fusarium wilt of watermelon is usually considered a monocyclic disease. Plant to plant spread does not occur because FON spores are usually produced later in the growing season and do not have the chance to infect other host plants in the same growing season. The disease can be spread in many ways. The disease may be spread as chlamydospores that are transported to other fields by infested tractors, operators, and tractor equipment, as well as rains from heavy floods. Fusarium wilt issues may also arise from using infected seed and/or transplants (Egel & Martyn, 2007).

To date, only the asexual stage (anamorphic) of *Fusarium oxysporum* has been observed; no sexual (teleomorphic) stage has been identified. *Fusarium* species are often referred to as anamorphic fungi due to their lacking a teleomorphic stage (Egel & Martyn, 2007). *Fusarium* species reproduce asexually by the production of macroconidia, microconidia, and/or sclerotia. Sporodochia, which is a mass of hyphae, produce macroconidia. When grown on agar plates these structures can be found on the center of the agar plate. These macroconidia are very characteristic for *Fusarium* species due to their banana or canoe-shaped spores. Each of these structures will be often be

segmented into 3-septated parts. Microconidia are much smaller than macroconidia and are often oval or kidney-shaped. They are typically aseptate and are formed on the aerial structures of mycelia (Kleczewski & Egel, 2011). This structure, as well as chlamydospores (thick walled spore), enable *F. oxysporum* to survive many years in an unfavorable environment (Komada, 1975).

F. oxysporum is a species that has over 100 members that have a special form. Each of these special forms has a particular host they infect. *Fusarium oxysporum* f. sp. *niveum* can only cause disease on watermelon (Zhou *et al.*, 2010; Kleczewski & Egel, 2011). However, it is possible that FON can infect other species, especially on younger plants but this has only been reported under greenhouse conditions (Zhou & Everts, 2007a). Some examples include melon (*Cucumis melo*), summer squash (*Cucurbita* spp.), as well as cucumber (*Cucumis sativus*) (Martyn & Mclaughlin, 1983; Zhou & Everts, 2007a). It can be very difficult and time consuming to distinguish the varying special forms of *F. oxysporum* as many special forms can be found in other non-hosts. Nonpathogenic *F. oxysporum* can infect watermelon but may classified as an endophyte due to special relationships formed within host plants (Malcom *et al.* 2012). *F. oxysporum* can live within the host plant and cause a systemic defense response rather than being pathogenic on its host (Larkin *et al.* 1996). Formae speciales that infect Cucurbits are a complex group as one formae speciales may be able to infect many genera with varying levels of pathogenicity. In contrast, some formae speciales use the same plants as hosts. Many efforts have been made to define the special forms as well as races within those special forms. Pathogenicity tests, vegetative compatibility tests, and

molecular techniques all attempt to differentiate the special forms as well as their respective races (Namiki *et al.*, 1994).

Fusarium oxysporum f.sp. of *niveum* also has four different races. These races can be determined by performing a pathogenicity test on a set of watermelon differentials (Table 1.1). In this set of tests, known cultivars of watermelons with varying levels of resistance are used to separate the race of the particular isolate being tested. Black Diamond or Sugar Baby are used as varieties that are susceptible to all races (0, 1, 2, and 3) of FON. Charleston Gray or Crimson Sweet are two varieties that can be used as indicators of resistance to race 0 but susceptibility to races 1, 2, and 3 (Zhou & Everts, 2003). Calhoun Gray or Allsweet are two cultivars that can be used to determine resistance to races 0 and 1, but are susceptible to races 2 and 3. PI-296341-*FR* is a differential resistant to races 0, 1, and 2 that can be used to identify a race 3 isolate of FON (Zhou *et al.*, 2010). For convenience, watermelon cultivars are simplistically coined either resistant or susceptible, however a range from highly susceptible to highly resistant can be used to describe each individual cultivar (Zhou & Everts, 2007b). Difficulties in race determination can be experienced by a multitude of factors including, but not limited to, inoculation techniques, inoculum concentration, environmental conditions, as well as the age of the inoculated plants (Zhou *et al.*, 2010).

The four different races of FON have varying levels of aggressiveness ranging from race 0 as the least aggressive to race 3 being the most aggressive. In the FON race complex, race is determined by virulence on a set of differential cultivars as opposed to being virulent on those same cultivars. It was not until 1972 that distinctions were made

between different isolates that had differences in virulence. Thus two different races were distinguished: races 0 & 1. By 1973, Netzer and Dishon discovered isolates that were virulent on race 1 resistant cultivar, and race 2 was assigned.

Race 1 isolates are the most commonly found isolates in the United States, however race 2 isolates have been found in many of the major watermelon producing areas of the United States (Zhou & Everts, 2003). States where race 2 isolates have been found include, but are not limited to, Texas, Oklahoma, Maryland, Delaware, Florida, Georgia, Indiana, and South Carolina. Race 2 isolates have also been reported in Cyprus, Greece, Spain, Turkey, and China (Zhou *et al.*, 2010). Race 2 prevalence is challenging to growers and scientists alike due to its more aggressive nature than races 0 and 1, and its difficult control (Zhou & Everts, 2003). Recently, race 3 isolates have been characterized from watermelon fields in Maryland. Race 3 differs from race 2 by being both more aggressive and virulent on race 2 determinate cultivars. It is thought that the emergence of race 3 isolates may have been due to selection pressure from monocropping race 1-resistant plants for up to 22 years (Zhou *et al.*, 2010). It was also shown by Hopkins et. al. 1992 that race 1-resistant cultivars such as Calhoun Gray and Dixielee lost resistance when monoculutred for five years or more and that proportions of race 2 isolates were increased (Hopkins *et al.*, 1992).

When testing for *F. oxysporum* either in soil or in plant tissue, Komada's medium is used to isolate the pathogen. Komada's medium was developed in 1976 as a way to select for and quantify *F. oxysporum*. L-asparagine and the acidification of the agar by the addition of phosphoric acid, enables colonies of *F. oxysporum* to develop a

characteristic pigmentation that sets them apart from other fungal colonies. Colonies that have a reddish-purple pigmentation as well as dense pinkish-white aerial hyphae are considered to be *F. oxysporum.* When quantifying colonies, characteristically colored colonies cannot be considered FON. Instead, all colonies must be counted as *F. oxysporum.* If further identification between pathogenic and nonpathogenic isolates is needed, a pathogenicity test is required. However, using the set of differentials to determine if an isolate is pathogenic and what race the isolate is, can be a time-consuming effort. Soil sampling for *F. oxysporum* can be used as a model to forecast disease severity (Komada, 1975).

In an attempt to categorize the formae speciales, scientists have used vegetative compatibility groups (VCG), isozyme profiles, as well as restriction fragment length polymorphisms (RFLPs). VCGs can be used to see how related isolates are to one another. If the isolates can form a heterokaryon with each other then they are filed into the same VCG. Because there has never been a sexual stage found in *F. oxysporum*, the interaction that occurs when forming a heterokaryon suggests that two species are genetically related. FON has been shown to have a strong correlation between its VCG and their pathogenicity (Namiki *et al.*, 1994).

Molecular approaches as well as a Fusarium database (FUSARIUM-ID) have been developed to decipher species of *Fusarium.* The translation elongation factor 1-alpha (TEF) is used to identify an isolate to the species level (Kleczewski & Egel, 2011). Polymerase Chain Reaction is used to amplify the TEF region by using both ef1 and ef2 primers. This amplified region of DNA is confirmed by band separation around 700

basepairs using gel electrophoresis. After editing of the DNA sequence, the isolates can be identified using the basic local alignment search tool (BLAST). This process can only be used to identify a known species or forma specialis (Kleczewski & Egel, 2011). Races of FON cannot be determined by molecular techniques (Zhou *et al.*, 2010). Koch's postulates must be used if a new species or race is found (Kleczewski & Egel, 2011).

Fusarium Wilt

Fusarium wilt was first observed in the United States in South Carolina and Georgia in 1894 (Zhou *et al.*, 2010). Symptoms of the disease are characterized at first by a dull green foliar chlorosis of the leaves and wilting of the entire plant or separate vines. Wilting may initially appear minimal and is followed by complete vine and plant collapse. In seedlings and young plants wilting or stunting may occur. In older plants disease is most severe at the fruit set stage. Wilting of individual runners as well as the entire plant is common. Necrotic lesions may be present in older parts of the plant that are near the soil line or just below the crown of the plant. These lesions may have hyphae growing from them in moist conditions (Kleczewski & Egel, 2011).

FON survives in the soil as chlamydospores for upwards of 15-20 years. When there is a susceptible host present, root exudates stimulate the chlamdyospores to germinate (Egel & Martyn, 2007). The mycelium penetrates the root-tip region via wounds and the radicle of germinating seeds and proceeds by invading the xylem tissue (Sitterly, 1972). It is here that FON produces microconidia that are transported via the vessels within the xylem. The microconidia intermittently germinate all along the xylem creating more mycelium and microconidia. The characteristic wilting seen in

watermelons is due to host plant response to infection. The plant, in response to the invading FON, will form tyloses or invaginations of the parenchyma tissue. These invaginations physically block the fungal tissue from being shuttled farther into the runner of the plant. The pathogen is restricted to the area below the tylose formation but the plant cannot get water to its extremities. In addition to the blockage of the xylem by the tylose formation, the parenchyma cells begin to break down which forms a gum. Together, the tylose formation and the gum cause a physical blockage of water to the vines on the plant. Due to this blockage, a plant may be only symptomatic on one runner or one side of the plant. When sections of diseased runners are cut, brown markings from the production of gums can be seen in the vascular tissue of the stem (Egel & Martyn, 2007). Optimal conditions for development of Fusarium wilt occurs when soil temperatures are between 25-27° C and there is low soil moisture (Kleczewski & Egel, 2011). If temperatures exceed 33° C, *Fusarium* will not develop (Sitterly, 1972).

Plants that can form tylose structures quickly enough to confine fungal propagules before they spread to other xylem tissue are termed resistant plants. Plants that do not form tyloses quickly enough and allow a systemic infection of FON to other xylem tissue are considered susceptible (Egel & Martyn, 2007). Diploid watermelons are usually more resistant to Fusarium wilt than are triploid varieties (Zhou *et al.*, 2010). Currently there is resistance to races 0 and 1 of FON but most varieties that have resistance to race 1 are diploid. However under high populations of FON in the soil, even race 1 resistance has the potential to break down (Egel & Martyn, 2007). There is no diploid or triploid variety that has resistance to races 2 & 3 (Keinath *et al.*, 2010). There are a few triploid

cultivars that are on the market that have moderate resistance to race 1(Zhou *et al.*, 2010). Due to the market demand, more and more acres of seedless (triploid) watermelons are being grown in the United States (Zhou & Everts, 2006). As South Carolina growers produce more acres of seedless watermelon the incidence of Fusarium wilt has become more common (Keinath & DuBose, 2009). This poses a problem as race 2 distribution spreads with no resistance genes in either diploid or triploid varieties (Zhou & Everts, 2006). Six of the eight top watermelon producing states in the US have reported race 2 in their watermelon fields, thus causing great concern for disease in the future (Zhou *et al.*, 2010). A grower also might have an issue with which cultivar to plant. For example: If a field is infested with race 2 FON and a race-1 resistant variety is planted, then the grower will still experience losses due to Fusarium wilt. The FON race present in a field would have to be known for resistant cultivars to be used efficiently (Keinath *et al.*, 2010).

'Fascination' is a high-yielding, seedless watermelon variety released by Syngenta. It has deep red flesh and matures 2 to 3 days earlier than that of Tri-X 313. 'Fascination' has improved, intermediate resistance to FON race 1. Tri-X 313 is another watermelon variety that is commonly grown due to its consistent yield and quality; however Tri-X 313 has no resistance to soil-borne pathogens (Anon. 2012).

Fumigants

Soil fumigation is a process by which a liquid is injected into the soil to provide some type of pest protection. Once the liquid is injected, it quickly volatizes and disperses as a gas. With over a century of research, soil fumigants have long been

studied for their biocidal effects on soil pests. Some fumigants have a broad-spectrum biocidal effect which kills fungi, nematodes, weeds, and many soil microflora. Other fumigants have a more targeted effect on one pest, such as nematodes, but can still provide some protection from weeds and fungi. Some popular fumigants of present and past include methyl bromide, chloropicrin, 1, 3-dichloropropene, and methyl isothiocyanate (Lembright, 1990).

These fumigants are injected into the soil as liquids through hoses that are attached to chisels. The chisels plow through the soil while the liquid fumigants are deposited into the furrow that the chisel creates (Lembright, 1990). The soil then closes back in on the liquid applied, using closing wheels, and is usually mulched with a plastic film (Lembright, 1990) (Rosskopf *et al.*, 2009). The plastic mulch serves to trap the gases that volatize from the liquid injected. The gases then diffuse into the soil air spaces in the bed that has been mulched. The efficacy of a fumigant is dependent on many factors (Lembright, 1990). While soil fumigation can help to minimize losses due to FON, it cannot be completely relied on as a management tool. The fumigant injected into the soil can only go so deep into the soil. This means that there are areas under the raised beds where the fumigant does not reach where the pathogen is still viable. When roots grow out of the fumigated area, they may become susceptible to FON causing late season disease (Egel & Martyn, 2007).

Methyl bromide has been widely used for many years in the agricultural sector as a fumigant injected into the soil as a pre-plant treatment to control various pests. Due to health and environmental concerns, methyl bromide has begun to be phased out all over

the world. Methyl bromide is classified under the Clean Air Act as a Class I ozone-depleting substance. It is regulated as a restricted use pesticide by the EPA's Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) as well as state regulation agencies. In 1987 the United States, as well as many other countries, signed The Montreal Protocol, an agreement to reduce the production and use of substances that deplete the stratospheric ozone. By 1992 the Copenhagen Amendment added methyl bromide to its list of ozone depleting substances. The Copenhagen Amendment allowed for counties to determine a baseline amount of methyl bromide used in the 1991growing season to serve as a starting point for the phase out. Each year since 1992 a gradual decrease in both the production and use of methyl bromide has occurred. Critical use exemptions (CUE) offer growers the opportunity to use methyl bromide under a highly regulated process. The exemption has to meet two criteria: "(i) The specific use is critical because the lack of availability of methyl bromide for that use would result in a significant market disruption; and (ii) there are no technically and economically feasible alternatives or substitutes available to the user that are acceptable from the standpoint of environment and public health and are suitable to the crops and circumstances of the nomination." In 2012, season critical use exemptions for cucurbits included the entire southeast. Fewer and fewer CUE's are being issued as more and more alternatives are being developed and implemented. The EPA received CUE's for 562,326 kilograms of methyl bromide in 2013, which is 2.2 % of the baseline amount from 1991, thus there is a greater need for alternative strategies to manage Fusarium wilt. In 2014 there will be no CUEs for Cucurbits (Anonymous 2012a).

Since the phasing out of methyl bromide began, new fumigant formulations have been researched in efforts to fill the niche of methyl bromide. One such new fumigant that could possibly replace methyl bromide is dimethyl disulfide. Paladin®, dimethyl disulfide (DMDS):chloropicrin, is a new fumigant that may be used on both vegetable and fruit crops in the future (Rosskopf *et al.*, 2009). Currently, there are no fumigants that can control FON spores as well as methyl bromide (Wechter *et al.*, 2012).

Managing Fusarium Wilt

Fusarium oxysporum f. sp. *niveum* causing Fusarium wilt is considered a serious pest. Managing Fusarium wilt can be a challenging obstacle when producing watermelon, as there is no one management technique that can be used that will sufficiently combat the disease. Instead, an integrated approach must be used to mitigate the losses that come from FON. While avoidance of FON is the best approach, many areas already have the pathogen present. It is critical to use disease-free seed and transplants when growing watermelons (Egel & Martyn, 2007). Integrated pest management techniques such as crop rotation, solarization, and cover crop use provide low efficacy against managing the pest. Fumigant soil disinfestation is considered a moderately effective method while grafting and host resistance are the best methods to manage Fusarium wilt, if the pathogen is already present (Louws *et al.*, 2010). If the race of FON is known and there is a resistant cultivar available, then that cultivar should be chosen (Keinath *et al.*, 2010). Crop rotations away from watermelons of 5-7 years can help with loses from Fusarium wilt by lowering the amount of pathogen inoculum;

however these rotations are usually not followed due to inadequate amounts of land (Zhou & Everts, 2007b). Even following years of rotation to a non-host crop, the pathogen still can have viable chlamydospores that will be able to infect the host plant although the inoculum level will not be as high (Egel & Martyn, 2007). It was shown by Zhou and Everts 2003 that when there is an increase of inoculum density, the rate of wilt also increases thus making inoculum density management a key component to controlling Fusarium wilt (Zhou & Everts, 2003). Even using multiple disease management plans, losses seen from Fusarium wilt still may occur (Egel & Martyn, 2007).

Soil solarization is also an approach that can be used to reduce the amount of FON in the soil. In this system, the soil is covered with plastic mulch and the soil below the plastic reaches temperatures high enough to kill the pathogen (Egel & Martyn, 2007). Temperatures that are necessary to kill fungal pathogens vary greatly and are dependent on many factors (Gamliel & Katan, 2012) . *F. oxysporum* propagules may be inactivated at 57.5-65°C for 30 minutes, however, this temperature may be found only in the top 5 cm of soil. Many times soil temperatures do not reach high enough levels below 30°C to inactivate fungal pathogens. As with crop rotations and fumigation, the pathogen still remains in the soil. Soil solarization is very laborious, expensive, and may not sufficiently control FON (Egel & Martyn, 2007).

Yet another approach to manage Fusarium wilt is the technique of grafting. Grafting has been used in the Middle East and East Asia for decades as a technique for disease management. In countries such as Israel, around 60-70 % of all watermelons are grafted (Cohen *et al.*, 2007). Although labor intensive, grafting provides many benefits

for both the grower and the plant. Plants that have been grafted often have more vigor due to enhanced water and nutrient uptake which results in a higher yield due the production of more photosynthates. Additionally, rootstocks can tolerate cooler or warmer temperatures and more adverse soil conditions than their scion counterpart, thus allowing watermelons to be grown in less desirable conditions. Perhaps the most intriguing aspect to grafting watermelons is disease protection that rootstocks can offer (Yetisir & Sari, 2003).

Literature Cited

Anonymous 2013. Health and Nutrition. *National Watermelon Promotion Board*. http://www.watermelon.org/HealthProfessionals/HealthProfessionals.aspx

Anonymous 2012a. Protection of Stratospheric Ozone: The 2013 Critical Use Exemption From the Phaseout of Methyl Bromide. *Federal Register :The Daily Journal of the United States Government.* 2013.

Anonymous 2012b. Vegetables 2011 Summary. *USDA,National Agricultural Statistics Service*.

Cohen R, Burger Y, Horev C, Koren A, Edelstein M, 2007. Introducing Grafted Cucurbits to Modern Agriculture - The Israeli Experience. *Plant Disease* 91, 916-923.

Egel DS, Martyn RD, 2007. Fusarium Wilt of Watermelon and Other Cucurbits. *The Plant Health Instructor*.

Gamliel A, Katan J, eds, 2012. *Soil Solarization Theory and Practice.* St. Paul, Minnesota: The American Phytopathological Society.

Hopkins DL, Lobinske RJ, Larkin RP, 1992. Selection for Fusarium-Oxysporum F Sp-Niveum Race-2 in Monocultures of Watermelon Cultivars Resistant to Fusarium-Wilt. *Phytopathology* 82, 290-293.

Keinath AP, Hassell RL, Everts KL, Zhou X, 2010. Cover Crops of Hybrid Common Vetch Reduce Fusarium Wilt of Seedless Watermelon in the Eastern United States. *Plant Health Progress* doi:10.1094/PHP-2010-0914-01-RS.

Keinath AP, DuBose V, 2009. First Report of Fusarium oxysporum f. sp niveum race 2 in South Carolina Watermelon Fields. *Phytopathology* 99, S63-.

Kleczewski NM, Egel DS, 2011. A Diagnostic Guide for Fusarium Wilt of Watermelon. *Plant Health Progress* doi:10.1094/PHP-2011-1129-01-DG.

Komada H, 1975. Development of a Selective Medium for Quantitative Isolation of Fusarium-Oxysporum from Natural Soil. *Review of Plant Protection Research* 8, 114-124.

Larkin RP, Hopkins DL, Martin FN, 1996. Suppression of Fusarium Wilt of Watermelon by Nonpathogenic Fusarium oxysporum and Other Microorganisms Recovered from a Disease-suppressive Soil. *Phytopathology* 86, 812-819.

Lembright HM, 1990. Soil Fumigation: Principals and Application Technology. *Supplement to Journal of Nematology* 22 (4S), 632-644.

Louws FJ, Rivarda CL, Kubotac C, 2010. Grafting Fruiting Vegetables to Manage Soilborne Pathogens, Foliar Pathogens,Arthropods and Weeds. *Scientia Horticulturae* 127, 127-146.

Malcolm GM, Kuldau GA, Gugino BK, Jimenez-Gasco MM, 2013. Hidden Host Plant Associations of Soilborne Pathogens: An Ecological Perspective. *Phytopathology* 103, 538-544.

Martyn RD, Mclaughlin RJ, 1983. Susceptibility of Summer Squash to the Watermelon Wilt Pathogen (Fusarium-Oxysporum F Sp-Niveum). *Plant Disease* 67, 263-266.

Namiki F, Shiomi T, Kayamura T, Tsuge T, 1994. Characterization of the Formae Speciales of Fusarium-Oxysporum Causing Wilts of Cucurbits by Dna-Fingerprinting with Nuclear Repetitive Dna-Sequences. *Applied and Environmental Microbiology* 60, 2684-2691.

Rosskopf EN, Kokalis-Burelle N, McSorley R, and Skvarch E, 2009. Optimizing Alternative Fumigant Applications for Ornamental Production in Florida. . *Florida Cooperative Extension Service ENY-901. http://edis.ifas.ufl.edu/in818.*

Rothrock CS, Kirkpatrick TL, Frans RE, Scott HD, 1995. The Influence of Winter Legume Cover Crops on Soilborne Plant-Pathogens and Cotton Seedling Diseases. *Plant Disease* 79, 167-171.

Sitterly W, 1972. Breeding for Disease Resistance in Cucurbits. *Annual Review of Phytopathology* 10:471-490.

Wechter WP, Kousik C, McMillan M, Levi A, 2012. Identification of Resistance to Fusarium oxysporum f. sp niveum Race 2 in Citrullus lanatus var. citroides Plant Introductions. *HortScience* 47, 334-338.

Yetisir H, Sari N, 2003. Effect of Different Rootstock on Plant Growth, Yield and Quality of Watermelon. *Australian Journal of Experimental Agriculture* 43, 1269-1274.

Zhou XG, Everts KL, 2006. Suppression of Fusarium Wilt of Watermelon Enhanced by Hairy Vetch Green Manure and Partial Cultivar Resistance. *Plant Health Progress* doi:10.1094/PHP-2006-0405-01-RS.

Zhou XG, Everts KL, 2003. Races and Inoculum Density of Fusarium oxysporum f. sp niveum in Commercial Watermelon Fields in Maryland and Delaware. *Plant Disease* 87, 692-698.

Zhou XG, Everts KL, 2004. Suppression of Fusarium Wilt of Watermelon by Soil Amendment with Hairy Vetch. *Plant Disease* 88, 1357-1365.

Zhou XG, Everts KL, 2007a. Characterization of a Regional Population of Fusarium oxysporum f. sp niveum by Race, Cross Pathogenicity, and Vegetative Compatibility. *Phytopathology* 97, 461-469.

Zhou XG, Everts KL, 2007b. Effects of Host Resistance and Inoculum Density on the Suppression of Fusarium Wilt of Watermelon Induced by Hairy Vetch. *Plant Disease* 91, 92-96.

Zhou XG, Everts KL, Bruton BD, 2010. Race 3, a New and Highly Virulent Race of Fusarium oxysporum f. sp niveum Causing Fusarium Wilt in Watermelon. *Plant Disease* 94, 92-98.

Table 1.1 Genotypes used to differentiate races of *Fusarium oxysporum* f. sp. *niveum*

	Race			
Genotype	**0**	**1**	**2**	**3**
Sugar Baby, Black Diamond	Susceptible	Susceptible	Susceptible	Susceptible
Charleston Gray, Crimson Sweet	Resistant	Susceptible	Susceptible	Susceptible
Calhoun Gray, Allsweet	Resistant	Resistant	Susceptible	Susceptible
PI-296341-*FR*	Resistant	Resistant	Resistant	Susceptible

CHAPTER TWO

COVER CROP STUDY

Introduction

Cover crops are crops typically grown before a cash crop that can provide benefits to both the soil and the grower. Benefits of growing cover crops can include protection from water and wind erosion, the addition of organic nitrogen and carbon, the enrichment of soil organic matter, the improvement of soil texture and physical properties, higher soil water retention and infiltration rates, weed and insect suppression, as well as increased soil microbial activity. All of these factors can contribute to the productivity of the soil on the following crop (Rothrock *et al.*, 1995; Nair & Ngouajio, 2012). The biomass produced from growing a cover crop will eventually make up the organic matter that will be added to the soil (Clark, 2007). The long-term goal of using cover crops is the build-up of organic matter in the soil. Organic matter improves the soil structure, increases water infiltration rates, conserves soil moisture, and serves as a long-term storage of nutrients. Additionally, organic matter keeps the soil active by providing simple sugars and proteins for soil microbes. These microbes can reduce plant disease by enhancing natural disease suppression mechanisms found in plants.

Due to their cold hardiness, winter cover crops are often chosen by growers to grow in the winter months before a summer cash crop is planted. Examples of commonly grown cover crops include hairy vetch, clover, rye, and other cereal crops (Clark, 2007). Legume crops such as clovers and vetches can provide nitrogen that will be available to vegetable crops such as watermelon at transplanting and throughout the season. The

amount of nitrogen provided to the system can range from 130 to 209 kg/ha (Hartwig & Ammon, 2002). In another study, hairy vetch provided 103 kg/ha per acre while rye, a non-legume cover crop, provided only 14 kg/ha per acre. Vetch also has fewer pest and disease problems than other cover crops (Shennan, 1992). Additionally, vetch can withstand lower temperatures than other cover crops. Vetch has been shown to increase a grower's net return on watermelon by $1974/ha per year when compared to a non-legume rye cover crop (Keinath *et al.*, 2010).

Hairy vetch can either be mowed and left on the surface of the soil or incorporated into the soil. When mowed and left on the surface of the soil as a mulch, hairy vetch can reduce both foliar and fruit diseases on tomato and pumpkin (Zhou & Everts, 2004). When incorporated into the soil, cover crops can control many soilborne pests such as *Pythium* spp., *Thielaviopsis* spp., *Fusarium* spp., and *Verticillium* spp.

When incorporated into soils, cover crops can either suppress or stimulate microorganism communities. Hairy vetch has been shown to suppress fungal propagules. The mechanism associated with the suppressive nature of the incorporation of hairy vetch is unknown; however it is thought that some suppression may be derived from volatile compounds produced by the hairy vetch. These compounds, such as ammonia, have been found in alkaline suppressive soils (Candole & Rothrock, 1997). When ammonium particles in the soil lose protons, ammonia is released from the system as a gas (Deacon, 2013). This gas, when trapped with plasticulture techniques, has fungicidal properties due to its ability to penetrate cell walls. The suppression of FON in field experiments with incorporated hairy vetch has been shown to be comparable to those seen when using

methyl bromide or other fumigants (Zhou & Everts, 2004; Zhou & Everts, 2006). Field study rates of about 0.2% (wt/wt) of hairy vetch led to considerable wilt reduction (45%) without the potential of ammonia phytotoxicity. In addition to reducing wilt, watermelon plants were more vigorous, had higher fruit yield, and had an increase in sugar content with hairy vetch incorporated into the soil when compared to the winter fallow control (Zhou & Everts, 2004). Zhou and Everts (2004) also found in greenhouse tests the incorporation of hairy vetch, urea, and crab shell suppressed Fusarium wilt. More specifically, hairy vetch reduced wilt symptoms 53 to 87% when compared to a control without a soil amendment. It is thought that bacteria in the soil play a role in wilt suppression. Wilt suppression has been correlated with an increase in populations of soil bacteria (Zhou & Everts, 2007). Zhou and Everts (2004) showed that a linear increase in ammonia production led to a linear decrease in soil populations of FON. The mechanisms behind the suppression are currently being studied; however the production of ammonia does not seem to be the only mechanism (Zhou & Everts, 2004).

In a 2007 study, Zhou and Everts demonstrated that using host resistance in watermelon in combination with a hairy vetch cover crop improved overall wilt suppression. However under high pathogen populations in the soil (> 1,100 colony-forming units) wilt suppression as well as a reduction in the pathogen population was not observed. As inoculum density increased, wilt suppression decreased (Zhou & Everts, 2007).

Although hairy vetch (*Vicia villosa*) has been shown to reduce Fusarium wilt, it is a host for the Southern root- knot nematode (*Meloidogyne incognita*). Keinath et al.

(2010) showed that a hybrid, 'Cahaba White' common vetch (*V. sativa* x *V. cordata*) is just as effective at suppressing Fusarium wilt when compared to hairy vetch and gives added resistance to the Southern-root knot nematode. This information could be utilized by growers that have Southern-root knot nematode problems (Keinath *et al.*, 2010).

Plastic Films

The use of films on raised beds has become a popular practice for commercial watermelon production. Blok et al. (2000) demonstrated that significant reductions in numbers of chlamydospores of *F. oxysporum* occur when an organic crop, such as broccoli or grass, are incorporated into the soil and tarped. The effectiveness of mulch depends on how long it prevents gases from escaping from the soil. Due to the phaseout of methyl bromide, efforts have been made to develop new and more improved films that are less permeable. The goal of this research has been to develop systems that help reduce methyl bromide emissions into the atmosphere. An ideal film should have the following characteristics: low permeability to gases, as well as high puncture and impact strengths. Low density polyethylene films (LDPE), the most commonly used film in agriculture, have excellent mechanical properties and provide a good barrier to water, but are not efficient in keeping gases trapped (Gamliel & Katan, 2012). LDPEs have been shown to lose anywhere from 30-80% of methyl bromide into the atmosphere after injection (Noling *et al.*, 2011-2012). Virtually impermeable film (VIF) is made with a barrier polymer (ethylene vinyl alcohol) which is sandwiched between polyethylene. This three-layered system provides a thicker film which allows for less gas to escape into the atmosphere (Anon., 1998). The ethylene vinyl alcohol layer (EVOH) provides an

excellent barrier that is extremely efficient in trapping gas. Gasses are less permeable even at temperatures ranging from 50-60° C when they would normally escape. Due to its chemical make-up, EVOH is water soluble and has to be layered in-between two layers of polyethylene (Gamliel & Katan, 2012). Totally impermeable film (TIF) is a five-layered, polyethylene-based film with a middle layer consisting of ethylene vinyl alcohol copolymer (EVOH). TIF films have a very low gas emission rate when compared to conventional LDPE. Because TIF films are not yet commercially available, there is little information on the efficacy of the new film (Gao *et al.*, 2011). Adding to their gas trapping properties, films can also be impregnated with metals, which leads to less permeability and a longer holding time of gasses in soil beds compared to more conventional LDPEs (Gamliel & Katan, 2012). An additional benefit of using thicker mulches is a more uniform distribution of gasses under the plastic (Gao *et al.*, 2011) Due to thicker films, an EVOH layer, and metals being impregnated into the film, less gas escapes from mulched beds with new plastic technologies. Growers can reduce the rates of fumigants, helping them economically as well as lessening ozone depleting gases escaping into the atmosphere by using newer plastic technologies (Gamliel & Katan, 2012). Conventional mulch, a black polyethylene (LDPE), has been used in previous tests to trap gasses produced by vetch breakdown, but no studies have been performed that evaluate the effectiveness of conventional mulch, VIF, or TIF to trap ammonia derived from vetch decomposition in one study (Zhou & Everts, 2004). As these technologies are developed, work needs to be done to evaluate their effectiveness with specific fumigants and organic amendments.

Soil Microorganisms

Incorporating a cover crop into the soil can increase the biodiversity and activity of the microbial community. Naturally suppressive soils in the Chateaurenard region of France and in the Salinas Valley of California both have a diversity of microorganisms including antagonistic bacteria and actinomycetes (Larkin *et al.*, 1996). Increased organic matter from incorporating cover crops increases general disease suppression. More specifically, the activity of fluorescent *Pseudomonas* and nonpathogenic *F. oxysporum* helps reduce disease by competing with pathogenic *F. oxysporum* for nutrients as well as infection sites on roots (Larkin *et al.*, 1996). In field studies where wilt was decreased, increased populations of *F. oxysporum* were seen in hairy vetch-amended fields (Zhou & Everts, 2006). It was proposed that the increased levels of nonpathogenic *F. oxysporum* as well as bacteria and other fungi played a role in suppressing disease.

Larkin et al. (1996) demonstrated that specific isolates of nonpathogenic *F. oxysporum* are responsible for suppression of Fusarium wilt of watermelon. These antagonistic isolates have very specific traits that other nonpathogenic *F. oxysporum* lack that enable them to provide disease protection to watermelon plants. These isolates provide a general level of disease suppression due to competition for resources, particularly carbon and iron. When specific nonpathogenic *Fusarium oxysporum* isolates are present, symptom development such as wilting in watermelon is delayed. Systemic infection by FON still occurs, but the onset of disease symptoms and the severity are

decreased. Many watermelon plants will exhibit only partial wilt on one side of the plant, suggesting that the pathogen is confined to one section of the vascular system. It is suggested that the mechanism of disease suppression involves constraining pathogen movement and colonization once inside the plant. (Larkin *et al.*, 1996).

Fluorescent *Pseudomonas* aid in controlling Fusarium wilt by competing for iron and by producing antifungal compounds (Larkin *et al.*, 1996). *Bacillus* spp. produce antibiotic and toxic substances which may impede FON development and growth (Blok *et al.*, 2000). Many species of bacteria that live in the rhizosphere, including *Pseudomonas* and *Bacillus* species, can induce a systemic response in host plants that strengthens a plant's defense system (Kloepper *et al.* 2004). Studies have shown that compounds such as peroxidase and isozymes are increased as a result of a mixture of beneficial bacterial species inducing a response within the plant. These defense compounds effectively reduce fungal colonization within a plant and aid in forming thicker cell walls to block out the pathogen from other cells. Many organic commercial products, such as Actinovate (Natural Industries, Houston, TX) use *Streptomyces* as their active ingredient to manage soilborne pests such as *Fusarium, Pythium, Phytophthora, Verticillium,* and *Rhizoctonia* (Actino-Iron, 2013).

Objectives

The objective of the cover crop study was to evaluate the efficacy of using hairy vetch and fumigants as management tools for Fusarium wilt. To achieve this goal, each treatment's impact on disease was measured by taking disease incidence ratings,

percentage area covered by healthy vines ratings, and an end of the year yield assessment. Pathogen isolations and pathogenicity testing was performed to confirm disease and identify pathogenic races of FON. Additionally, mechanisms thought to aid in suppression of disease were assessed by performing quantitative soil assays on microbes and ammonia concentrations.

Materials and Methods

The field studies were performed in Charleston, SC at Clemson University's Coastal Research and Education center in a field with naturally occurring populations of FON races 1 and 2 (Keinath et al. 2010). The field had previously been cropped to a cultivar of watermelon susceptible to FON. The design of the experiment was a Latin square with five treatments and five replications per treatment. The length of each plot was 21.33 meters in 2011-2012 and 15.24 meters in 2012-2013 with a 1.52-meter non-planted space between plots.

On 27 October 2011 and 18 October 2012, a cover crop was planted in each of the 25 plots. Three treatments were seeded with hairy vetch at 112.06 kg/ha and two treatments were seeded with Abruzzi rye at 67.25 kg/ha in 2011. In the 2012 experiment, three treatments were seeded with Abruzzi rye, one treatment was seeded with hairy vetch, and one treatment was seeded with a combination of the two at the same rates as 2011. Due to insufficient germination, the rye plots were re-seeded on 1 November 2012 at 67.25 kg/ha. The vetch and rye combined plot was not re-seeded to ensure the vetch was not disturbed. A biomass measurement of all the cover crops was taken on 7 March

2012 and 28 February 2013 before they were sprayed with herbicide, mowed, and disked into the soil; the disk being washed between each treatment. Wild *Brassica* spp. were weeded as needed due to their ability to produce isothiocyanates. The soil then was bedded and covered with film. One vetch treatment was covered with LDPE film, another vetch treatment was covered with VIF plastic film, and the last vetch treatment was covered with TIF plastic film in 2011-2012. One rye treatment was covered with VIF plastic while the other rye treatment received dimethyl disulfide + chloropicrin (467.69 L/ha) injected as a fumigant and covered with VIF in both 2011-2012 and 2012-2013. In addition to the dimethyl disulfide + chloropicrin treatment, there was another fumigant treatment, methyl bromide + chloropicrin (538 kg/ha), as well as a vetch and a vetch + rye treatment in 2012-2013; all using VIF. The rye treatment served as the negative control in both field experiments.

On 7 Mar 2012 the rye plots were mowed and on 5 Mar 2013 the rye plots were mowed and disked. Gramoxone (3507 ml/ha) was sprayed on the vetch plots on 19 Mar 2012. Both the vetch and rye plots were disked on 19 and 22 Mar 2012 and 6, 8, and 13 March 2013. The rye plots received 560 kg/ha of 15-0-15 (N-P-K) in 2012 and 466 kg/ha with an additional 15 kg/ha of 0-0-22 fertilizer in 2013. The vetch plots were estimated to have 93 kg/ha organic nitrogen so they received 316 kg/ha of 15-0-15 (N-P-K) and 24 kg/ha of 0-0-22 (N-P-K) fertilizer to ensure the vetch plots received equal amounts of nitrogen and potassium as the rye plots in 2012. Nitrogen estimates were made based on vetch providing 4% and rye providing 2% nitrogen content from their respected dry weight. In 2013, the vetch treatment was estimated to have 68 kg/ha of

nitrogen therefore 15-0-15 was added at 475 kg/ha and 0-0-22 fertilizer was added at a rate of 12 kg/ha. It was estimated that the vetch + rye treatment added 76 kg/ha of nitrogen per acre so an additional 427 kg/ha of 15-0-15 as well as 20 kg/ha of 0-0-22 fertilizers were added to the treatment. Dual Magnum (1555 ml/ha) and Ridomil Gold (2338 ml/ha) were sprayed on 29 Mar 2012 and Sandea (73 ml/ha) was sprayed 20 Mar 2013 before beds were pressed and plastic was laid. In 2012 three types of plastic films were used, LDPE, VIF, and TIF, while in 2013 only VIF was used.

The cultivar of seedless watermelon used was 'AC 7187,' which is susceptible to FON. On 7 Mar 2012 and 20 Feb 2013, 'AC 7187' was seeded in the greenhouse. 'AC 7187' watermelons were transplanted 1.22 meters apart within rows on 19 Apr 2012 and 16 Apr 2013. In 2012 'SP5' pollenizers were transplanted in-between every fourth seedless 'AC 7187' plant while in 2013 'SP6' pollenizers were used.

Throughout the 2012 growing season, insects were managed with one application each of Actara (219 ml/ha) and Provado (511 ml/ha), two applications of Fulfill (201 ml/ha) and Brigade (468 ml/ha), and three applications of Lannate SP (1.12 kg/ha). One application each of Actara (219 ml/ha), Venom (292 ml/ha), and Agrimek (256 ml/ha), and three applications of Brigade (468 ml/ha) were used in 2013 to manage insects. In 2012, foliar diseases were managed with six applications of Procure (585 ml/ha), two applications each of Presidio (292 ml/ha) and Previcur Flex (1403 ml/ha), and one application each of Quintec (438 ml/ha), Folicur (585 ml/ha), Tanos (586 ml/ha), and Fontelis (1169 ml/ha). Due to rainy conditions during the 2013 growing season, many foliar diseases were managed with one application each of Monsoon (585 ml/ha), Inspire

Super (1169 ml/ha), Previcur Flex (1403 ml/ha), Dithane Rainshield (5612 ml/ha), Presidio (292 ml/ha), Pristine (1315 ml/ha), and two applications of Bravo (2338 ml/ha). Additionally 2338 ml/ha of Ridomil was applied through the drip tape to manage oomycetes in the soil. Weeds were managed with Prowl H_2O (2455 ml/ha) and Gramoxone (3508 ml/ha) in 2013.

Biomass and Soil Sampling

Cover crop plant material above the soil line was collected from two 0.25 m^2 areas in each plot using an electric hedger on 7 March 2012 and 28 February 2013. The fresh plant material was collected in paper grocery bags, weighed, and oven dried at 100 C for 48 hours in the 2012 study. Plant material was oven dried at 100 C for 96 hours in 2013. The dried material was weighed and recorded.

Soil samples were taken in each of the plots on 19 December 2011 and 10 January 2013 after the cover crops had been established. Each sample consisted of ten cores of soil, mixed thoroughly, from a depth of 0-20 cm. This depth was chosen because 81% of *F. oxysporum* is found from this depth according to Zhou and Everts (2004). On 14 May 2012, after the cover crops had been incorporated, sampling of soil for FON included samples from 0-10 cm as well as samples from 10-20 cm. Samples that were taken at two depths were also repeated on 17 April and 14 May 2013. Colonies of *F. oxysporum* were grown on Komada's Fusarium-selective medium (see technique below) and quantified (Komada, 1975). Due to varying moisture levels in the soil, ten grams of soil were weighed from each plot and put into a small envelope to dry out the soil. The

envelopes were put into an oven (Precision Economy Oven, Precision Scientific, Inc.) and allowed to dry for six days. The dry weight of each sample was recorded and *F. oxysporum* colony counts were adjusted based on the dry weight of each individual sample. In addition to culturing FON, cultures of fluorescent *Pseudomonas, Streptomyces,* and *Bacillus* were prepared from soil samples from the rhizosphere of one randomly selected watermelon plant per plot. Rhizosphere samples used for quantifying populations of bacteria were taken on 9 May 2012 and 28 May 2013.

Technique for quantifying Fusarium

a) Ten core samples of 0-10 cm and/or 10-20 cm were taken from each plot and mixed thoroughly

b) Ten grams of soil was mixed with 90 ml of sterile deionized water

c) Samples were vortexed for 2 minutes and allowed to settle (10^{-1} dilution)

d) One ml of solution was taken and added to 9 ml of sterile deionized water (10^{-2} dilution)

e) One-tenth of one milliliter of 10^{-1} sample was added onto Komada's Fusarium-selective medium (10^{-2} dilution) and 0.1 ml of 10^{-2} sample was added onto Komada's Fusarium-selective medium (10^{-3} dilution) (Komada, 1975)

f) Suspensions were spread on agar with a glass "Y"-shaped rod

g) Plates were allowed to incubate for 8 to 10 days at room temperature (22-24 C) and 16 hour daylength

i) Colonies of Fusarium-like (pink) color were counted for both dilutions

Technique for quantifying bacteria

a) One randomly selected watermelon plant from each plot was dug and the soil was shaken from the roots. Roots were placed in a plastic bag and transported to the lab to be processed.

b) A 1 g sample of the roots and adhering rhizosphere soil was cut and placed in 99 ml of sterile deionized water the same day samples were collected

c) Serial dilutions were prepared as follows: *Bacillus* 10^{-5} and 10^{-6}, *Streptomyces* 10^{-5} and 10^{-6}. Fluorescent *Pseudomonas* were plated using the 10^{-4} and10^{-5}dilutions in 2012 and 10^{-3} and 10^{-4} in 2013

c) One-tenth of one milliliter of dilution was plated on separate media for *Bacillus* (1/10 TSA), *Streptomyces* (M3), fluorescent *Pseudomonas* (S1)

d) The 10^{-3} and 10^{-4} *Bacillus* dilutions were heated in an 80 C water bath for 10 minutes before plating to enrich the species population (Keinath, 1996.)

d) Suspensions were spread using a "Y"-shaped rod and incubated at 22-24 C

e) An estimation of *Bacillus* were made 7 days after plating, an estimation of *Streptomyces* were made 7 days after planting, and an estimation of *Pseudomonas* were made 2 days after plating (Keinath, 1996.)

Determining Ratio of Pathogenic to Nonpathogenic Colonies of *F. oxysporum* from Soil Dilutions

Ten soil samples were taken from each individual plot at two different depths (0-10 cm and 10-20 cm) on 17 April 2013. Soil was mixed thoroughly and 10 grams of soil was added to 90 ml water. Samples were vortexed for 2 minutes and allowed to settle (10^{-1} dilution). 1 ml of solution was taken and added to 9 ml of distilled water (10^{-2} dilution). 0.1 ml of 10^{-1} sample was added onto Komada's Fusarium-selective medium (10^{-2} dilution). 0.1 ml of 10^{-2} sample was added onto Komada's Fusarium-selective medium (10^{-3} dilution) (Komada, 1975). Suspensions were spread on agar with a glass "Y"-shaped rod. Two plates per dilution were prepared. Plates were allowed to incubate for 8 to 10 days at room temperature (22-24 C) and 16 hour daylength. Colonies of Fusarium-like (pink) color were counted for both dilutions. Four colonies characteristic of *Fusarium oxysporum* appearance were selected from the 0-10 cm depth and four were selected from the 10-20 cm depth to serve as the isolates used for the quick pathogenicity testing (described in Chapter 3). A total of 40 isolates from each treatment were used in the cover crop experiment. Twenty isolates came from each of the following treatments: the rye control treatment, the dimethyl disulfide + chloropicrin treatment, and the methyl bromide + chloropicrin treatment. A quick pathogenicity test was performed as described in Chapter 3, with the only exception being a sterile needle was used to collect spores from chosen colonies that were on the Komada's medium. The spores were added to a mineral salts broth. After performing a quick pathogenicity test on each isolate, a ratio of pathogenic to nonpathogenic *F. oxysporum* was determined.

Ammonia Sampling

An ammonia trapping system, as described by Zhou and Everts (Zhou & Everts, 2004) was used to measure the amount of ammonia produced by the breakdown of vetch by each field treatment. In 2012, fourteen days post-incorporation of cover crops, a plastic centrifuge tube consisting of 30 ml of a 2% boric acid solution was prepared. The tube containing the 2% boric acid was capped until it was ready to be put out into the field. The tube was then covered with two layers of cheesecloth and a rubber band just before it went into the field. The plastic film was cut with a razor blade to reveal a flap to insert the tube. A soil probe was used to make a hole for the tube to slide into the soil. The centrifuge tube was inserted into each hole and the flap was covered and taped shut with duct tape to ensure that no gasses could escape. Twenty-four hours after the tubes were buried; they were collected and brought back to the lab for ammonia analysis. This process was repeated again 28 days after cover crop incorporation. The Paladin treatments did not have ammonia readings at fourteen days post-incorporation but did have a 28 day reading in 2012. A positive control was established by adding 0.1 kg of 46-0-0 (N-P-K) fertilizer to a 0.25 m^2 plot covered with VIF film to ensure that ammonia was being collected by the centrifuge tubes. In the 2013 season the process was repeated with the only difference being only one sampling was performed at 21 days post incorporation.

Ammonia concentrations were determined following a test kit procedure (Vacu-vials K-1403 CHEMetrics Inc, Calverton, VA). In this test kit method, ammonia

undergoes a series of reactions with salicylate and experiences a color change depending on the ammonia concentration (Zhou & Everts, 2004). Because the ammonia samples were in boric acid, the pH had to be adjusted to 12 to change the trapped ammonium to ammonia. Therefore, 1.8 ml of 5 N NaOH was added to each of the 25 samples. Five drops of A-1402 Stabilizer Solution was added to an empty sample cup. Next the ammonia samples with the adjusted pH were filled to the 25 ml mark in the sample cup. Two drops of A-1401 Catalyzer Solution was added, stirred, and followed by the addition of two drops of A-1400 Activator Solution. The contents were mixed and an ampoule was quickly broken off in the sample jar which allowed the sample to be taken up into the ampoule. The sample was then mixed by rocking the ampoule side to side for ten seconds and left for at least fifteen minutes. Samples that turned a deep blue had an ammonia value far exceeding the range of the test kit (0.20 to 3.00 ppm). Based on previous readings it was determined that the dilution range should be 25-375 ppm for the urea plots. Five drops of A-1402 Stabilizer Solution was added to an empty sample cup. 200 µl of the adjusted pH ammonia sample was added to the sample cup. The sample cup was then filled with 23.4 ml of 2% boric acid. To adjust the pH of the sample, an additional 1.6 ml of 5 N NaOH was added to the sample cup. Next, the Catalyzer and Activator solutions were added and the ampoule was broken as described before.

Once the vials had reacted for fifteen minutes and undergone their color change, a 3 ml disposable hypodermic syringe was used to extract the contents of each ampoule. The liquid was then transferred to an empty 96 well ELISA plate. Each well in the ELISA plate received 250 µl of the ammonia sample and was repeated into six wells. In

addition to the 25 samples from the field, an adjusted pH tube of 2% boric acid and 1.8 ml of 5 N NaOH and a blank tube from the test kit were also tested for absorbance to serve as standards.

The ELISA plates were then read using a spectrophotometer (Molecular Devices Spectra Max Plus 384 Spectrophotometer). The samples were read at 610 nm wavelength and absorbance values were given for each sample. The average of the six samples from each ampoule were taken and fit into a standard curve line of best fit values established for ammonia concentrations (Krom, 1980). This procedure was repeated in 2013 with the only exception being only one sampling was taken; 21 days post incorporation of cover crops.

Mini Urea Experiment

Previously bedded and pressed soil was chosen as the site for the urea and mulch experiment. The design of the experiment was a completely randomized block with four replications and five treatments. The five treatments are as follows: totally impermeable film (TIF) with added urea, virtually impermeable film (VIF) with added urea, conventional film (LDPE) with added urea, a no mulched plot with added urea, and a VIF plot with no urea added. The bare beds were marked off 0.61m by 1.66m and 0.198 kg of urea (41-0-0) was evenly spread over each bed by hand on 7 Jun 2012. The urea was then turned into the soil with a hand cultivator. Next, the three types of film were hand cut, laid, and covered depending on the treatment. The procedure used for collecting and determining the ammonia levels under each plastic was the same as described before.

However, there was only one ammonia sampling at twenty-four hours after incorporation of urea which occurred on 15 Jun 2012.

Full concentration samples were performed on the no mulched treatments with urea added and also on the VIF film and no urea added treatments due to their ammonia concentration ranging from 0.20 to 3.00 ppm. The concentrations of ammonia from the LDPE, VIF, and TIF films with urea added were higher than the range of the test kit therefore a dilution had to be performed as described above.

Disease Progression and Harvest

Plants with Fusarium wilt symptoms (stunting, yellowing, wilted vines, and/or dead vines) were assessed weekly starting 3 May 2012, two weeks post-transplant, and continued until 27 Jun 2012. These plants were marked with a wooden stake with the date they were found diseased or dead and the type of symptom that was first seen. The number of dead, diseased, and healthy plants was recorded each week until the first harvest of the season. Scouting for Fusarium wilt began on 1 May 2013, two weeks post transplant, using the same rating procedure as described in 2012. Plants exhibiting non-typical symptoms were selected at random throughout the entire growing season to confirm Fusarium wilt within the field. Some examples of the non-typical symptoms included: dead leaves but healthy vines, non-vigorous plants, stunting, and partial wilt. In addition to the disease incidence ratings, a percentage area covered rating was taken in both field experiments. This scale uses the most healthy plot as a standard in which to rate the other plots based on percentage of healthy foliage. This rating can be correlated

to the amount of disease found in each plot as the same cultivar, 'AC 7187,' was used throughout the study. The percentage area covered ratings were taken on 8 and 27 June 2012, as well as 30 May and 19 June 2013. Marketable and wildlife damaged watermelons were harvested when ripe and weighed once a week for four weeks on 2, 9, 16, and 23 July 2012 and 28 June 3, 10, and 17 July 2013 to obtain a total marketable yield for the field season. Ripened small, sunburned, and deformed watermelons were not measured as part of the study.

Pythium and *Fusarium* Sampling

Stunted plants were observed in the field on 25 May 2012. One stunted plant from each treatment was selected for root and stem sampling. The roots would give an indication if *Pythium* spp. and/or *Fusarium oxysporum* were infecting the plant while the stem samples would suggest *F. oxysporum* was present. The stems of the diseased plants were cross sectioned with a razor blade a few centimeters above the soil line. The cross sectioned stems were surface-disinfested in a 0.6% sodium hypochlorite solution for 1.5 minutes. The stems were rinsed in sterilized distilled water for 30 seconds and then blotted until dry. The stems were cut into four 0.2 x 0.1 cm sections and placed on Komada's *Fusarium*-selective medium and left in the light to incubate for 5 days. Colonies of *F. oxysporum* were identified based on formation of salmon-colored colonies. Small pieces, 0.5 cm to 1.0 cm, of discolored root tissue were cut from the diseased plants. The diseased roots were surface disinfested in a 0.3% sodium hypochlorite solution for 30 seconds, rinsed, and blotted. Roots were placed onto water

agar + streptomycin (100 mg/L) medium and allowed to incubate in the light for 1-3 days. *Pythium* and *Fusarium* colonies were observed under a dissecting microscope and recorded. This procedure was repeated for plants exhibiting non-typical symptoms in 2013. In addition to the May 2012 stunted plant sampling, another sampling was performed on 16 July 2012. Many of the plots suddenly collapsed within one week's time. Two plants from each plot that had collapsed were sampled and tested for *Pythium* and *F. oxysporum* in 2012.

Statistical Analysis

All data was analyzed using SAS software package (version 9.2 and 9.3; SAS Institute Inc., Cary, NC). Analysis of variance was performed using PROC MIXED or PROC GLM on data and means were compared using the Shiparo-Wilke least significant difference at $P = 0.05$. Ammonia concentrations were square root transformed before being analyzed due to non-normally distributed data in 2012. Bacteria data were transformed using the log or log + 1 transformation. Prior to analysis, disease incidence data was transformed using the arcsine square root transformation. Contrast analyses were performed to examine any differences among groups of treatments. Additionally, a repeated measures analysis was used to examine differences in CFUs over time. Area under the disease progress curve was calculated by the sum PROC means and then analyzed by PROC GLM.

Results

Fusarium oxysporum colony-forming units (CFU) in soil

In the January 2012 sampling, there were no differences in the colony-forming units (CFU) of *F. oxysporum* among the five treatments with either dilution. In the May sampling, after the cover crops had been incorporated into the soil, there was a significant effect of sampling depth ($P<0.0001$) on CFUs. The 0-10 cm depth had a mean of 41.09 CFUs/ 10 g soil while the 10-20 cm sampling depth had a mean of 25.08 CFUs/ 10 g soil. There was also a highly significant effect among treatments; however there was no interaction between treatment and depth. All three vetch treatments had significantly ($P<0.05$) higher CFUs than did the rye or the fumigated treatment in the 10^{-2}/ 10 g soil dilution at a depth of 0-10 cm in May (Table 2.1). The rye treatment and dimethyl disulfide + chloropicrin treatment had similar CFUs to each other in the May, 0-10 cm sampling depth. The CFUs were at least doubled from the January to the May sampling in all vetch treatments in the 10^{-2}/ 10 g soil dilution at a depth of 0-10 cm. Dimethyl disulfide + chloropicrin was the only treatment that numerically, but not statistically, lowered CFUs of *F. oxysporum* from the January to the May sampling, at both sampling depths; in all other treatments CFUs increased. The fumigant was statistically different ($P<0.05$) from all three vetch treatments in its difference in CFUs between January and May soil samplings. CFUs increased significantly in all vetch treatments. The rye treatment also increased CFUs however, not statistically different from the fumigant treatment.

In 2013 there were no differences in the CFU's in the January sampling prior to incorporation or fumigation (Table 2.2). However, CFUs in both the 0-10 cm and 10-20 cm depths differed among treatments in the April and May sampling. The fumigated plots, dimethyl disulfide + chloropicrin and methyl bromide + chloropicrin, had significantly lower populations of *F. oxysporum* than the other three treatments in the April sampling, four weeks post fumigation, at both depths. In the May sampling, eight weeks post fumigation, methyl bromide + chloropicrin had significantly lower CFUs than the rye, vetch, and vetch + rye treatments. The two fumigant treatments both significantly reduced the CFUs from January to April and maintained low CFUs in the May sampling. Additionally, when grouped together in a contrast analysis, both fumigants had lower CFUs when compared to the control (non-fumigated rye) in both the April ($P<0.0001$) and May ($P=0.0074$) samplings when the depth data was combined. The rye and vetch + rye had similar patterns during the samplings; the CFUs were comparable in both January and April and significantly decreased in the May sampling. Rye and vetch + rye treatments, when grouped together in a contrast analysis, had significantly lower CFUs ($P=0.0153$) than the vetch treatment in May. The vetch treatment significantly increased the CFUs from January to April, and significantly decreased CFUs from April to May. Unlike the 2012 sampling, in the 2013 sampling there was no effect of sampling depth on CFUs in either April or May ($P=0.339$).

Colony-forming units of *Bacillus,* fluorescent *Pseudomonas,* and *Streptomyces*

Bacterial populations surrounding the rhizosphere of watermelon plants were sampled once watermelon roots had established six weeks post-transplanting. In the 2012 and 2013 samplings there were no differences in CFU/g rhizosphere soil among treatments for *Bacillus* spp. or *Streptomyces* (Tables 2.3 & 2.4). However in the 2013 sampling, CFUs of fluorescent *Pseudomonas,* in the rye control and the methyl bromide + chloropicrin treatment were lower ($P<0.05$) than in the vetch + rye treatment. Fluorescent *Pseudomonas* populations were statistically similar among treatments in 2012.

Biomass and ammonia assays

Average fresh weight biomass of cover crops did not differ among treatments in the 2011-2012 experiment (Table 2.5). In the 2012-2013 experiment, two out of the three rye treatments had a significantly higher fresh weight biomass than vetch alone (Table 2.6). Biomass of one of the rye treatments was similar to the vetch + rye treatment, while the vetch treatment alone had a significantly lower biomass than the other two rye treatments. The average soil incorporation rate (wt/wt) ranged from 0.61 % to 1.37 % in both years.

Boric acid was buried under VIF, TIF, and LDPE film at 7 and 20 days in each treatment to determine the amount of ammonia produced by the cover crops and the amount of ammonia trapped by the individual films. The 2012 seven-day sampling revealed that there was no significant difference between the three films in the ability to

trap ammonia (Table 2.7). However, in the 20-day sampling vetch with LDPE film produced a significantly higher concentration of ammonia than the rye control. In 2013, ammonia was sampled, seven days after mulch was laid, and no differences in the ammonia concentrations were seen between the rye, vetch or vetch + rye treatments (Table 2.8).

A "mini" film trial was set up to determine if there were any differences in the ammonia-trapping abilities of VIF, TIF, and conventional LDPE film (Table 2.9). There were no statistical differences in the concentrations of ammonia trapped by of any of the films or the controls. However, the three films with added urea trapped more and similar amounts of ammonia compared to mean of the no-urea and no-film treatments (P=0.068). This confirms that the films trapped ammonia that was produced when urea was added before mulching.

Percentage area covered and disease incidence ratings

Two separate ratings were taken during the growing season, which compared the percentage area covered by vines in individual plots to the percentage area covered in the plot with the healthiest and most vigorous vines. In both ratings in 2012, a vetch with conventional film plot (LDPE) (Replication 2, Treatment 5, Row 1) served as the standard to which all other plots were compared. In 2013, methyl bromide + chloropicrin plots were chosen as the standard to compare all other plots (Replication 3, Treatment 4, Row 4 and Replication 1, Treatment 4, Row 1 for ratings one and two respectively). In both experiments, there were no statistical differences among any

treatments at either rating. The initial ratings in the 2012 season were higher than the initial ratings in the 2013 season. However, in 2012 the percentage area rating decreased from the first rating to the second rating (Table 2.10). The biggest change in percentage area rating was seen in the dimethyl disulfide + chloropicrin treatment, which decreased by 30%. In comparison, the rye with VIF only changed 16% and the combined vetch averages only changed 15.8%. With the 2013 percentage area ratings, all plots had a higher percentage rating in the second rating than the first; the only exception being dimethyl disulfide + chloropicrin, which decreased in rating by 4% (Table 2.11). In both years dimethyl disulfide + chloropicrin had a lower rating at the second rating date than the first.

A disease progress curve was created to track the progress of Fusarium wilt on a week-to-week basis throughout the growing season. In both field experiments, a general trend of disease progression was found. In both years, disease symptoms were found three weeks post-transplanting. After initial disease was noted, there was a plateau where very few additional symptomatic plants were seen for a few weeks. However, after this plateau there was a spike in disease incidence starting in the sixth week of disease rating (or seven weeks post-transplanting), which continued until the last rating week just before the first harvest. In the 2012 season disease was severe, and ranged from 61-70% of plants that were diseased (Table 2.13). These plants exhibited typical symptoms of Fusarium wilt of watermelon, including wilting, dead vines, stunted growth, yellowing, and entirely dead plants. All treatments followed a similar trend in disease progression with dimethyl disulfide + chloropicrin suppressing Fusarium wilt slightly longer than

other treatments in 2012, until rating week 6 (Figure 2.1). After rating week 6, disease progression of the treatments that were fumigated appeared similar to the other treatments. There were no significant differences among treatments in disease progression in the 2012 field season. Disease pressure was lower in 2013 than 2012; the most diseased treatment exhibited and average of 21.67% of plants showing Fusarium wilt symptoms (Table 2.14). There were no significant differences in disease progression at any point throughout the 2013 growing season. The area under the disease progress curve (AUDPC) was similar among treatments in both field seasons. The AUDPC was higher in 2012 with the average of the treatments being 1107 while in 2013 the average was only 500. There were no significant differences in AUDPC among treatments in either the 2012 or the 2013 experiments (Table 2.12 & 2.14).

A wilt suppression index (WSI) was calculated to determine the effectiveness of the cover crops, plastic films, and the fumigants (Table 2.12 & 2.14) (Zhou & Everts, 2007). The range of the WSI was from 0 (no suppression) to 100 (complete suppression). The WSI of the combined vetch averages from 2012 was 8.06 % while the fumigant dimethyl disulfide + chloropicrin was 6.33 % and the rye control was 0. In the 2013 study, neither vetch nor vetch + rye provided any wilt suppression with a WSI of 0. Fumigants, methyl bromide + chloropicrin and dimethyl disulfide + chloropicrin, provided some wilt suppression in 2013. Dimethyl disulfide + chloropicrin had a 13% WSI while methyl bromide + chloropicrin provided 27% wilt suppression.

Watermelon harvest

In 2012 there were no significant differences in yield of marketable watermelons per treatment. Marketable yield from the 2012 season was lower when compared to the 2013 season (Table 2.15 & 2.16). The marketable weight ranged from 13,664 kg/ha to 16,940 kg/ha while the number of marketable watermelons ranged from 20 to 25.2 per plot. Higher yields were seen in the 2013 season with methyl bromide + chloropicrin yielding 30,044 kg/ha as well as the highest number of marketable watermelons. The weight and number of watermelons produced per plot using methyl bromide + chloropicrin was significantly higher than using vetch alone. Based on the Waller-Duncan k-ratio *t* test there were no differences in yields among other treatments.

Sampling for *Fusarium oxysporum* and *Pythium* in diseased plants

In both field experiments unhealthy plants that had non-typical Fusarium wilt symptoms were sampled and cultured onto either water agar + streptomycin and/or Komada's selective medium to test for either *Pythium* or *Fusarium oxysporum.* On the last sampling in 2012, plants were sampled in plots that had collapsed within one week of the last sampling; one rating week they were healthy and by the next rating week they were either dead or close to dying. In total 17/40 pieces of tissue sampled from the roots of diseased plants contained *F. oxysporum* and 13/28 pieces contained *Pythium* (Table 2.17). Seventy-nine percent of stem pieces that were plated onto Komada's selective media were confirmed as *F. oxysporum.* Only one plant out of fifteen sampled

throughout the season did not have at least one piece of tissue that was positive for *F. oxysporum*.

In 2013 every plant sampled, 14 total, contained *F. oxysporum* (Table 2.18). In the root tissue samples, 37% of the pieces of tissue sampled had *F. oxysporum* while 80% contained *Pythium.* Ninety-one percent of the stem tissue samples contained *F. oxysporum.* In both sampling years, a higher percentage of *F. oxysporum* was found in the stem of the plant as compared to the root tissue.

In the quick pathogenicity study to determine the ratio of pathogenic to non-pathogenic *F. oxysporum* from soil dilutions, it was found that 2 out of 120 isolates collected caused disease on Sugar Baby. One of the isolates came from the rye control plot at a depth of 10-20 cm while the other came from the methyl bromide + chloropicrin treatment at the 0-10 cm sampling depth. Six seeds were sown for each isolate and a total of nineteen seeds did not germinate on a total of 17 isolates. The race 2 reference isolate and the two possible-pathogenic isolates were recovered on Komada's medium.

Discussion

Managing Fusarium wilt has been a challenge for many years around the world. Much research has led to several management practices that aim to control the disease. One such management practice involves incorporating a cover crop of vetch into the soil. In studies where hairy vetch reduced Fusarium wilt, attempts have been made to explain the mechanisms that are thought to be involved in the suppression. Some mechanisms involved in suppression include increased populations of nonpathogenic *F. oxysporum,*

increased antagonistic bacterial populations, and increased ammonia concentrations (Larkin *et al.*, 1996; Zhou & Everts, 2004). In my experiments, I examined each of these proposed mechanisms and their influence on the reduction of Fusarium wilt. Another technique used to manage Fusarium wilt is the use of pre-plant fumigants to control inoculum levels of FON (Zhou & Everts, 2004). I examined changes in CFUs over time when fumigants were used. I also tracked percentage area covered, disease incidence, and yield and how each was impacted by either the vetch or fumigant treatments.

It has been shown many times by Zhou and Everts that incorporating a cover crop of vetch can significantly reduce Fusarium wilt in Maryland (Zhou *et al.*, 2003; Zhou & Everts, 2004; Zhou *et al.*, 2004). In one particular study the wilt percentage for the rye control was 73% while the vetch treatment was 56% (Zhou & Everts, 2007). In all of the studies there was a significant difference between the control treatments and the vetch in wilt percentage; however, the wilt percentage in the vetch treatments were still relatively high (Zhou *et al.*, 2003; Zhou & Everts, 2004; Zhou *et al.*, 2004). In a similar study in South Carolina, rye and vetch treatments did not differ in Fusarium wilt percentage at two different rating dates, with the cultivar '7167,' which has no Fusarium wilt resistance (Keinath & Hassell, 2008). In a study that compared wilt percentage after the incorporation of hairy vetch in both Maryland and South Carolina, there were differences in wilt percentage data between the two states. The wilt suppression index (WSI) for hairy vetch in South Carolina was 18 % and 4 % and the WSI in Maryland was 32 % and 43 % in 2012 and 2013, respectively (Keinath *et al.*, 2010). The combined hairy vetch

average wilt suppression in my study was only 8 % in 2012 and 0 % (no wilt suppression) in 2013.

Previous studies have shown that incorporating vetch can increase total populations of *F. oxysporum* which could potentially increase populations of specific nonpathogenic *F. oxysporum.* These forms of *F. oxysporum* colonize roots and play a role in wilt suppression by inducing a systemic defense response in watermelon plants (Larkin *et al.*, 1996). In the vetch treatments, *F. oxysporum* populations in the soil significantly increased from the baseline sampling to the samplings later in the year done after incorporation of vetch. In 2013, 118 out of 120 colonies from soil dilution samples did not cause disease on watermelon seedlings in a quick pathogenicity test. A high nonpathogenic to pathogenic ratio of *F. oxysporum* suggests that there are more nonpathogenic *F. oxysporum* competing for resources in the soil and on the roots compared to *F. oxysporum* f. sp. *niveum.* Such a low population of FON would make it more difficult for the pathogen to cause disease than if the ratio were reversed. Due to low spore counts in later quick pathogenicity experiments with other isolates, it cannot be concluded that these 118 out of 120 isolates were nonpathogenic; some isolates may not have produced enough spores to effectively colonize and cause disease in watermelon seedlings. More than two of the isolates could possibly be pathogenic, but more pathogenicity tests would have to be performed. Although colonies were randomly selected from the soil dilution plates, pathogenic colonies that may have existed may not have been chosen. Due to low disease pressure in 2013, it is not surprising that so few pathogenic colonies were found. Increased populations of nonpathogenic *F. oxysporum*

have been shown to delay the onset of Fusarium wilt; however, this was not observed in my two-year study (Larkin *et al.*, 1996).

It has also been suggested that some Fusarium wilt suppression comes from antagonistic bacteria in the soil. Beneficial bacteria, *F. oxysporum,* and many other microbes compete for the same resources in the rhizosphere. Beneficial bacteria produce antifungal compounds to help them compete for these resources (Larkin *et al.*, 1996). Some bacteria can colonize the root tips, the zone of elongation, and the bend in the roots and induce systemic resistance and alter root exudates (Ling *et al.*, 2012). These functions of bacteria may play a role in suppressing the pathogen from attacking the roots of the watermelon. Previous work has shown that bacteria populations, fungal populations, and some actinobacteria populations increased significantly after hairy vetch was incorporated. As bacterial populations increase, wilt suppression increases (Zhou & Everts, 2007). In my study I examined the differences among *Bacillus*, fluorescent *Pseudomonas*, and *Streptomyces* populations in the rhizosphere of plants in each of the treatments to see if population differences occurred based on treatment. Populations of all three groups were similar among treatments post-incorporation, and due to disease incidence being very similar in all treatments, it is difficult to either prove or disprove the hypothesis that bacteria play a role in suppressing Fusarium wilt. Field soil was sampled at four weeks post-incorporation of 1% wt/wt of hairy vetch by Zhou and Everts (2007). Field soil did not come directly from the rhizosphere of the watermelon plant and was sampled closer to the incorporation date than in my study. Competition between microbes occurs in the rhizosphere, therefore soil samples should be taken from the

rhizosphere to better capture what happens closest to the plant roots. Samples should be taken once roots have grown out of the transplanting media to ensure that bacteria from field soil are colonizing the rhizosphere. It can be concluded under the experimental conditions in my study that vetch does not increase populations of *Bacillus* or *Streptomyces* in the rhizosphere at six weeks post incorporation.

High ammonia concentrations produced from the breakdown of leguminous vetch have been shown to suppress Fusarium wilt by reducing FON populations (Zhou & Everts, 2004). It has been proposed that ammonia toxicity can have adverse effects on chlamydospore viability of *Thielaviopsis basicola* at concentrations ranging from 0.075 and 0.1 parts per million (ppm) (Candole & Rothrock, 1997). However, with certain fungi such as *Fusarium solani* f. sp. *phaseoli*, ammonia concentrations under 50 ppm can stimulate germination of conidia (Pavlica *et al.*, 1978). The concentrations of ammonia detected in the vetch plots were all under 1 ppm. Ammonia concentrations were similar in both 2012 and 2013. In my seven-day sampling, the concentrations were similar to those reported in Candole and Rothrock (1997). With minute and statistically similar ammonia concentrations, as well as higher CFUs in vetch treatments, ammonia toxicity was not a mechanism that suppressed Fusarium wilt in my field studies Additionally, ammonia was not the mechanism that increased CFUs of *F. oxysporum* in vetch treatments. In greenhouse experiments when ammonia concentrations reached levels upwards of 140 ppm using hairy vetch, urea, and crab shell, significant wilt reduction was observed (Zhou & Everts, 2004). Concentrations of 163 and 520 ppm ammonia generated from using 1% wt/wt and 5 % wt/wt urea, respectively, in greenhouse

experiments would be very difficult to produce in a field setting. The breakdown of a cover crop and its ammonia concentration in soil could be dependent on the soil type (Clark, 2007). Organic matter is broken down at varying rates depending on the soil type, microbial composition, and soil structure. In my 'mini' film trial, a 0.1 % rate of urea generated a maximum of 4.77 ppm ammonia.

There were no differences in the ability of the films to trap ammonia. This study, however, is not a sufficient determination of the trapping capabilities of each film. With minute quantities of ammonia that were all under 5 ppm, it was difficult to determine which film was better in its trapping ability. More tests measuring higher levels of a gas other than ammonia at different intervals of time would be the direction for future work. It cannot be concluded from this study if any one particular film is better in a watermelon production system to manage Fusarium wilt.

Another management tool that has been used by growers around the world to manage Fusarium wilt is the use of pre-plant fumigants such as methyl bromide (Cohen *et al.*, 2007). Fumigants work due to their nonspecific, toxic nature to all living things in the soil (Lembright, 1990). When fumigants are used to manage Fusarium wilt, the fumigant reduces *F. oxysporum* propagules in the soil, including both pathogenic and nonpathogenic forms. In my study, both fumigants significantly reduced the number of CFUs of *F. oxysporum* from the baseline determined in the January 2013 sampling. This suggests that there are biocidal properties of both fumigants that neither the control rye treatment nor the vetch treatments could provide under my experimental conditions. In Maryland, 1,3-dicholorpropene + chloropicrin, methyl bromide, methyl bromide + vetch,

and metam sodium all significantly reduced CFUs when compared to the non-amended control or vetch treatments (Zhou & Everts, 2004). The hairy vetch and fumigant treatments also significantly increased yield and significantly reduced wilt when compared to the non-treated control (Zhou and Everts 2004). The reduction in CFUs from their studies mirrors my results. In my study there was no interaction between sampling depth and treatment, suggesting that both fumigants work equally as well in the range of 0-20 cm where more than 81% of *F. oxysporum* is found (Zhou & Everts, 2004). It can be concluded that fumigants such as dimethyl disulfide + chloropicrin and methyl bromide + chloropicrin reduce CFUs of *F. oxysporum* from 0-20 cm in the soil profile. Due to less inoculum in the soil, plants should face less disease pressure in fumigated soil, as there is a strong correlation between inoculum density and wilt percentage (Zhou & Everts, 2003). Although there may be less inoculum in the soil, it is possible that disease incidence may not be reduced as pathogenic isolates may still exist. One pathogenic isolate was found in the methyl bromide + chloropicrin treatment in the soil dilution quick pathogenicity test. If roots come into contact with the pathogen, no matter the inoculum level, disease symptoms will develop. This could happen over time as the biocidal effects of the fumigant wears off or as roots grow out of the fumigated regions (D. S. Egel and R. D. Martyn., 2007.). There were no significant differences in disease incidence among any treatments in my study, although CFUs were significantly lower in the fumigated treatments.

Percentage area covered ratings were higher initially in 2012 than the ratings were in 2013. The 2013 spring was unusually cold and was accompanied with above normal

amounts of rain. These conditions led to slower plant development than in 2012, which may explain why the ratings were initially higher in 2012. At the second rating time, all treatments declined in their rating in 2012 while in 2013 only one treatment showed a decline. This could be explained by more optimal growing conditions after the initial slow start by the plants in 2013 as well as less disease pressure. From the percentage area covered ratings in 2012, it can be concluded that there was a higher disease pressure in 2012. Because the same cultivar, 'AC 7187,' was used in the cover crop study, the percentage area covered rating was directly correlated to disease severity. The area that was not covered by foliage was attributed to Fusarium wilt, as some plants had died or severely wilted which made the percentage area covered rating drop. All percentage area covered ratings were lower in the last rating in 2012 which suggests that the decline was due to Fusarium wilt.

Although disease percentage was higher in 2012, the disease progression in both years followed a similar trend. Initially there was very little disease until the plants became established three weeks post-transplant. There was a plateau of very little disease and then plants became very susceptible to Fusarium wilt. Symptoms of Fusarium wilt are generally seen 3-4 weeks post-transplant and then later around fruit set; symptoms were experienced at both of these growth stages in 2012 and 2013 (Kleczewski & Egel, 2011). This pattern has been observed in many studies by Zhou and Everts. In such studies symptoms develop slowly but become more "apparent toward harvest (Zhou *et al.*, 2002)."

Similar studies in Maryland and South Carolina have given rise to conflicting data on suppression of Fusarium wilt. In the Maryland studies, incorporating vetch into the soil suppresses Fusarium wilt; vetch does not have the same effect under experimental conditions in South Carolina (Zhou *et al.*, 2004; Keinath & Hassell, 2008; Keinath *et al.*, 2010). In future studies, wilt ratings should be taken until the first harvest date to examine the effects of vetch on wilt suppression. In previous work in Maryland, ratings were taken up until one week before harvest, and there was a significant reduction in wilt (Zhou *et al.*, 2003; Zhou & Everts, 2004; Zhou *et al.*, 2004). However, in one particular study final disease ratings were taken at 45 or 51 days after transplanting (Zhou & Everts, 2004). In my study I performed a percentage area covered rating on entire plots which directly correlates to the disease severity in the plots. This rating also allows the researcher to quantify the health and vigor of entire plots. In addition, when noting disease incidence, a severity rating should be taken on individual plants. A plant that has one wilted or dead vine near harvest will still produce watermelons that are of marketable size, but a plant that is completely wilted or dead will produce small, unmarketable, or no watermelons at all. A rating scale that examines how diseased a plant is would give a more accurate description of what is happening within the treatments (Ling *et al.*, 2012). Perhaps disease incidence is high but the plants are still healthy enough to produce a crop. Rating plants for disease incidence and disease severity until a set developmental stage and calculating a disease index would give researchers more Fusarium wilt disease data for future studies.

Low yields were seen in 2012 compared to 2013. In 2012, there was a higher disease percentage across all treatments. Weakened, stunted, unhealthy, and dead plants all contributed to the low yield. Additionally, many 'SP5' pollenizers died early in the season, which caused very little fruit to set as flowers are produced after Fusarium wilt is seen (Gunter & Egel, 2012). In 2013, the methyl bromide + chloropicrin treatment produced a significantly higher marketable yield than did the vetch treatment. The reduction in CFUs in the fumigated treatment allowed plants to produce a higher marketable yield than the vetch treatments. Using vetch as a cover crop increased yield in some studies while in other studies, control plots yielded a similar marketable yield as a vetch cover crop. Due to this variability in yield data, vetch is not guaranteed to increase yields (Zhou, Everts, and Armentrout 2003; Zhou and Everts 2004; Zhou, Everts, and Armentrout 2004; Zhou *et al.*, 2002; Keinath & Hassell, 2008).

The two rye treatments in 2013 had a statistically higher biomass than did the vetch. The lower biomass produced from the vetch treatment in both 2012 and 2013 conflicts with data published by Keinath et. al (2010). In their work, the vetch biomass was greater than the rye biomass. In their study, a seeding rate of 35.94 kg/ha for rye and 88.73 kg/ha for hairy vetch was used while I used 67.39 kg/ha for rye and 112.32 kg/ha for hairy vetch. In other similar Fusarium wilt studies in Maryland, hairy vetch was seeded at rates ranging from 44.92-50.54 kg/ha. Lower seeding rates were used in Maryland but a slightly higher biomass was found than in my study ranging from 4717 kg/ha to 7188 kg/ha dry weight; in my study a two-year average dry weight biomass was found to be 4043 kg/ha (Zhou *et al.*, 2003; Zhou & Everts, 2004; Zhou *et al.*, 2004). In a

three-year study evaluating the effect of cover crops in organic vegetable production, Nair and Ngouajio found that there were no differences in biomass between a rye cover crop and a vetch + rye cover crop in two out of the three years of the study (Nair & Ngouajio, 2012). Although vetch was seeded at a higher rate compared to rye in my study, rye produced a higher biomass in 2013. This may be attributed to the rye plant being a denser and stiffer plant compared to the fragile and vine-like nature of the vetch.

From the two year field study evaluating varying films, fumigants, and cover crops as management tools for Fusarium wilt, a few conclusions can be drawn. Hairy vetch increased CFUs of *F. oxysporum* while methyl bromide + chloropicrin and dimethyl disulfide + chloropicrin reduced CFUs. No combinations of vetch, varying films, or any fumigants were shown to reduce disease incidence in either field experiment. Additionally, vetch did not increase yields. In the current watermelon production system under my experimental conditions, it can be concluded that there is no benefit as to using hairy vetch, alone, as a cover crop for Fusarium wilt management. However, this was only a two-year study and more benefits may come from long-term use of cover crops as they increase soil organic matter and provide energy for beneficial soil microbes.

In Maryland, cover cropping and incorporating hairy vetch into the soil is a proven Fusarium wilt management tool; however, the same results have not been observed in South Carolina. Explanations as to why suppression is achieved in Maryland but not South Carolina may be due to many sources of variability among field sites. Sources of variability can include (but are not limited to): percentage of soil organic

matter, soil type, pathogen populations, beneficial populations of soil microbes, cultural practices, and weather conditions. Perhaps varying field conditions, prior to the incorporation of hairy vetch, were different between the two study sites. An example could include the percentage of organic matter, as it can differ greatly among different soil types (Clark, 2007). Different soil types have different rates at which they break down organic matter. The percentage of organic matter in the soil prior to the incorporation of hairy vetch was not measured in my study and not presented in the Maryland studies. Organic matter offers many benefits to the soil including providing simple sugars and proteins that microbes use for energy (Clark, 2007). Beneficial soil microbe populations may have been initially higher in Maryland due to a different percentage of organic matter and soil type. Additionally, more specific isolates of beneficial *F. oxysporum* may have been present or affected more by hairy vetch incorporation in Maryland than in South Carolina. An array of fungicides were used to manage foliar pests in both Maryland and South Carolina studies. If the fungicides came into direct contact with the organisms in soil, or inside the plant tissue, it could have altered the relationship between the beneficial microbe and the host plant. Perhaps the fungicides had more of an adverse effect on beneficial soil microbes in South Carolina than in Maryland. An herbicide was used to kill the hairy vetch before it was incorporated into the soil in my study. The herbicide could have changed the chemical structure of the killed cover crop, which could have had an effect on the soil organic matter, soil microbes, and watermelon plant. There are many sources of variability that

exist when performing field studies that could explain the lack of Fusarium wilt suppression in South Carolina.

Currently there is not a single management tool that can be relied on completely to manage Fusarium wilt. Instead, Fusarium wilt must be managed by integrating many practices, techniques, and technologies (D. S. Egel and R. D. Martyn, 2007). A system must be designed for each situation on an individual farm. Many factors must be taken into consideration when preparing for Fusarium wilt management when growing a watermelon crop (D. S. Egel and R. D. Martyn, 2007). One must know the history of a field before deciding to plant watermelon (Zhou & Everts, 2003). If a field had severe Fusarium wilt problems in the past, the grower may want to consider another crop, as neither resistance nor growing a cover crop can help in suppressing wilt (Zhou & Everts, 2006). If a field has had few Fusarium wilt problems or has been out of watermelon production for a few years, then a watermelon crop could be considered. Knowing the history of a watermelon production field can also aid the grower in choosing a specific cultivar (Zhou & Everts, 2003). For example, if a field has had very low disease pressure in the past, then a cultivar with no resistance or race 0 resistance may be sufficient. However, if a field has had severe disease pressure in the past, then a more resistant cultivar or the use of grafted plants may be an option for a grower (D. S. Egel and R. D. Martyn, 2007). When a cultivar that is moderately resistant to race 1 is grown, there is an additive effect on disease suppression than when a cultivar with no resistance is grown (Zhou *et al.*, 2003; Zhou & Everts, 2004) Combining a resistant cultivar of watermelon, along with using hairy vetch, can supplement Fusarium wilt management, however, this

was not part of my study and further studies need to be done to confirm this management technique (Zhou & Everts, 2004).

Literature Cited

Anonymous. 2013. Actino-Iron. http://naturalindustries.com/commercial/index.php/index.php?option=com_content&view=article&id=9&Itemid=9.

Anonymous. 1998. Controlling Methyl Bromide Emissions with Impermeable Films. *USDA, ARS, Products and Services.* *http://www.ars.usda.gov/is/np/mba/april98/films.htm*.

Blok WJ, Lamers JG, Termorshuizen AJ, Bollen GJ, 2000. Control of Soilborne Plant Pathogens by Incorporating Fresh Organic Amendments Followed by Tarping. *Phytopathology* 90, 253-259.

Candole BL, Rothrock CS, 1997. Characterization of the Suppressiveness of Hairy Vetch-amended Soils to Thielaviopsis basicola. *Phytopathology* 87, 197-202.

Clark A, ed, 2007. *Managing Cover Crops Profitably., Handbook Series Book 9.* . Beltsville, MD: Sustainable Agriculture Network.

Cohen R, Burger Y, Horev C, Koren A, Edelstein M, 2007. Introducing Grafted Cucurbits to Modern Agriculture - The Israeli Experience. *Plant Disease* 91, 916-923.

D. S. Egel and R. D. Martyn., 2007. Fusarium Wilt of Watermelon and Other Cucurbits. *T. he Plant Health Instructor. DOI: 10.1094/PHI-I-2007-0122-01*.

Deacon J, 2013. The Nitrogen Cycle and Nitrogen Fixation. *The Microbial World* Institute of Cell and Molecular Biology. The University of Edinburgh.

Gamliel A, Katan J, eds, 2012. *Soil Solarization Theory and Practice.* St. Paul, Minnesota: The American Phytopathological Society.

Gao S, Hanson BD, Wang D, Brownie GT, Quin R, Ajwa H, and Yates SR, 2011. Methods evaluated to Minimize Emissions from Preplant Soil Fumigation. *California Agriculture* 65(1):, 41-46.

Gunter C, Egel DS, 2012. Staminate Flower Production and Fusarium Wilt Reaction of Diploid Cultivars Used as Pollenizers for Triploid Watermelon. *HortTechnology: Preliminary and Regional Reports* 22, 694-699.

Hartwig NL, Ammon HU, 2002. 50th Anniversary - Invited article - Cover Crops and Living Mulches. *Weed Science* 50, 688-699.

Keinath AP, 1996. Soil Amendment with Cabbage Residues and Crop Rotation to Reduce Gummy Stem Blight and Increase Growth and Yield of Watermelon. *Plant Disease* 80:, 564-570.

Keinath AP, Hassell RL, 2008. On-farm Evaluation of Hairy Vetch and Fumigation for Integrated Control of Fusarium Wilt on Seedless Watermlon, 2008. *Plant Disease Management Reports* 3:V035.

Keinath AP, Hassell RL, Everts KL, Zhou X, 2010. Cover Crops of Hybrid Common Vetch Reduce Fusarium Wilt of Seedless Watermelon in the Eastern United States. *Plant Health Progress* doi:10.1094/PHP-2010-0914-01-RS.

Kleczewski NM, Egel DS, 2011. A Diagnostic Guide for Fusarium Wilt of Watermelon. *Plant Health Progress* doi:10.1094/PHP-2011-1129-01-DG.

Klopper, J.W., Ryu, C., Zhang, S., 2004. Induced Systemic Resistance and Promotion of Plant Growth by *Bacillus* spp. The Nature and Application of Biocontrol: *Bacillus spp.* *Phytopathology* 94, 1259-1266.

Komada H, 1975. Development of a Selective Medium for Quantitative Isolation of Fusarium-Oxysporum from Natural Soil. *Review of Plant Protection Research* 8, 114-124.

Krom MD, 1980. Spectrophotometric Determination of Ammonia: A Study of a Modified Berthelot Reduction Using Salicylate and Dichloroisocyanurate. *The Analyst* 105, 305-316.

Larkin RP, Hopkins DL, Martin FN, 1996. Suppression of Fusarium Wilt of Watermelon by Nonpathogenic Fusarium oxysporum and Other Microorganisms Recovered from a Disease-suppressive Soil. *Phytopathology* 86, 812-819.

Lembright HM, 1990. Soil Fumigation: Principals and Application Technology. *Supplement to Journal of Nematology* 22 (4S), 632-644.

Ling N, Zhang W, Tan S, Huang Q, Shen Q, 2012. Effect of the Nursery Application of Bioorganic Fertilizer on Spatial Distribution of Fusarium oxysporum f. sp niveum and its Antagonistic Bacterium in the Rhizosphere of Watermelon. *Applied Soil Ecology* 59, 13-19.

Nair A, Ngouajio M, 2012. Soil Microbial Biomass, Functional Microbial Diversity, and Nematode Community Structure as Affected by Cover Crops and Compost in an Organic Vegetable Production System. *Applied Soil Ecology* 58, 45–55.

Noling JW, Botts DA, MacRae AW, 2011-2012. Alternatives to Methyl Bromide Soil Fumigation for Florida Vegetable Production. *IFAS Extension. University of Florida* Chapter 6.

Pavlica DA, Hora TS, Bradshaw JJ, Skogerboe RK, Baker R, 1978. Volatiles from Soil Influencing Activities of Soil Fungi. *Phytopathology* 68, 758-765.

Rothrock CS, Kirkpatrick TL, Frans RE, Scott HD, 1995. The Influence of Winter Legume Cover Crops on Soilborne Plant-Pathogens and Cotton Seedling Diseases. *Plant Disease* 79, 167-171.

Shennan C, 1992. Cover Crops, Nitrogen Cycling, and Soil Properties in Semiirrigated Vegetable Production Systems. *HortScience* 27, 749-754.

Zhou XG, Everts KL, 2004. Evaluation of Hairy Vetch Cover Crop, Cultivar Resistance and Fumigation for Management of Fusarium Wilt and Anthracnose of Watermlon, 2004. *Plant Disease Management Reports* 20:V003.

Zhou XG, Everts KL, 2007. Evaluation of Resistance Inducers and Cover Crop for Management of Fusarium Wilt in Triploid Watermelon, 2007. *Plant Disease Management Reports* 2:V074.

Zhou XG, Everts KL, 2006. Suppression of Fusarium Wilt of Watermelon Enhanced by Hairy Vetch Green Manure and Partial Cultivar Resistance. *Plant Health Progress* doi:10.1094/PHP-2006-0405-01-RS.

Zhou XG, Everts KL, Armentrout DK, 2002. Hairy Vetch and Urea Soil Amendments: A Novel Stragety for Management of Fusarium Wilt in Watermelon, 2002. *Plant Disease Management Reports* 18:V006.

Zhou XG, Everts KL, Armentrout DK, 2003. Management of Fusarium Wilt of Triploid Watermelon with Hairy Vetch Cover Crop and Cultivar Resistance, 2003. *Plant Disease Management Reports* 19:V002.

Zhou XG, Everts KL, Armentrout DK, 2004. Evaluation of Hairy Vetch Cover Crop, Tillage and Plastic Mulch for Suppression of Fusarium Wilt of Watermelon, 2004. *Plant Disease Management Reports* 20:V004.

Zhou XG, Everts KL, 2003. Races and Inoculum Density of Fusarium oxysporum f. sp niveum in Commercial Watermelon Fields in Maryland and Delaware. *Plant Disease* 87, 692-698.

Zhou XG, Everts KL, 2004. Suppression of Fusarium Wilt of Watermelon by Soil Amendment with Hairy Vetch. *Plant Disease* 88, 1357-1365.

Zhou XG, Everts KL, 2007. Effects of Host Resistance and Inoculum Density on the Suppression of Fusarium Wilt of Watermelon Induced by Hairy Vetch. *Plant Disease* 91, 92-96.

Table 2.1 Cover crop treatments 2012 & 2103

2012 Treatments			2013 Treatments		
Cover Crop	**Plastic Film**	**Fumigant**	**Cover Crop**	**Plastic Film**	**Fumigant**
Rye	VIF[x]	None	Rye	VIF	None
Rye	VIF	Dimethyl disulfide + chloropicrin[y]	Rye	VIF	Dimethyl disulfide + chloropicrin
Vetch	VIF	None	Rye	VIF	Methyl bromide + chloropicrin[z]
Vetch	TIF	None	Vetch + Rye	VIF	None
Vetch	LDPE	None	Vetch	VIF	None

[x] Plastic films used were as follows: virtually impermeable film (VIF), totally impermeable film (TIF), and low density polyethylene film (LDPE).
[y] A 79:21 rate of dimethyl disulfide + chloropicrin was used for fumigation.
[z] A 50:50 rate of methyl bromide + chloropicrin was used for fumigation (Terr-O-Gas).

Table 2.2 Comparison of colony-forming units (CFU) of *Fusarium oxysporum* from soil dilutions in January and May using various cover crops, plastic films, and dimethyl disulfide + chloropicrin, 2012

Treatment							
Cover Crop	**Plastic Film[w]**	**Fumigant**	**January *Fusarium oxysporum* CFU X 10^{-2}/10 g soil at 0-20 cm depth[x]**	**May *Fusarium oxysporum* CFU X 10^{-2}/10 g soil at 0-10 cm depth**	**May *Fusarium oxysporum* CFU X 10^{-2}/10 g soil at 10-20 cm depth**	**May *Fusarium oxysporum* CFU X 10^{-2}/10 g soil combined depth (0-20 cm)**	**Difference in CFUs from May to January**
Rye	**VIF**	**None**	16.35 a[y]	28.24 c	20.50 ab	24.37 cd	8.02 bcd
Rye	**VIF**	**Dimethyl disulfide + chloropicrin[z]**	22.08 a	20.92 c	12.78 b	16.85 d	-5.23d
Vetch	**VIF**	**None**	20.49 a	44.44 b	23.02 ab	33.73 bc	13.24 abc
Vetch	**TIF**	**None**	23.61 a	56.19 a	39.00 a	47.59 a	23.99 a
Vetch	**LDPE**	**None**	24.46 a	55.67 a	30.15 ab	42.91 ab	18.44 ab

[w] Plastic films used were as follows: virtually impermeable film (VIF), totally impermeable film (TIF), and low density polyethylene film (LDPE).

[x] All colony counts are shown as CFU X 10^{-2}/10 g soil.

[y] Means within the same column followed by the same letter are not significantly different, Waller-Duncan k-ratio *t* test, k=100 or *P*=0.05.

[z] A 79:21 rate of dimethyl disulfide + chloropicrin was used for fumigation.

Table 2.3 Comparison of colony-forming units (CFU) of *Fusarium oxysporum* from soil dilutions in January, April, and May using various cover crops and fumigants, 2013

Cover Crop[u]	Fumigant	January *Fusarium oxysporum* at 0-20 cm depth[v]	April *Fusarium oxysporum* at 0-10 cm depth	April *Fusarium oxysporum* at 10-20 cm depth	April *Fusarium oxysporum* combined depths	May *Fusarium oxysporum* at 0-10 cm depth	May *Fusarium oxysporum* at 10-20 cm depth[w]	May *Fusarium oxysporum* combined depths
Rye	**None**	63.26 a A[x]	57.25 a	69.76 a	63.50 a A	44.06 b	38.01a	36.63 b B
Rye	**Dimethyl disulfide + chloropicrin[y]**	64.44 a A	20.77 b	21.53 b	21.15 b B	22.44 cd	16.60 b	19.52 c B
Rye	**Methyl bromide + chloropicrin[z]**	57.93 a A	11.13 b	5.83 b	8.48 b B	18.58 d	4.91b	9.89 c B
Vetch + Rye	**None**	62.64 a A	63.86 a	63.63 a	63.75 a A	37.53 bc	40.84 a	39.18 b B
Vetch	**None**	59.06 a B	77.41 a	73.17 a	75.29 a A	63.02 a	51.31 a	57.16 a B

[u] All treatments used virtually impermeable film (VIF) as the plastic film.
[v] All colony counts are shown as CFU X 10^{-2}/10 g soil.
[w] There were no significant differences in depth between the April and May samplings (P=0.339).
[x] Means within the same column followed by the same lower case letter are not significantly different and means within the same row followed by an upper case letter are not significantly different,Waller-Duncan k-ratio *t* test, k=100 or P=0.05.
[y] A 79:21 rate of dimethyl disulfide + chloropicrin was used for fumigation.
[z] A 50:50 rate of methyl bromide + chloropicrin was used for fumigation (Terr-O-Gas).

Table 2.4 *Bacillus, Streptomyces* and fluorescent *Pseudomonas* colony-forming units from soil dilutions, 2012

Treatment					
Cover Crop[w]	**Plastic Film**	**Fumigant**	***Bacillus* spp. CFU X 10^{-5}/g rhizosphere**	***Streptomyces* spp. CFU X 10^{-5}/g rhizosphere**	**Fluorescent *Pseudomonas* CFU X 10^{-4}/g rhizosphere**[x]
Rye	**VIF**	**None**	9.10 a[y]	20.20 a	0.16 a
Rye	**VIF**	**Dimethyl disulfide + chloropicrin**[z]	13.70 a	26.80 a	0.55 a
Vetch	**VIF**	**None**	12.50 a	61.30 a	0.98 a
Vetch	**TIF**	**None**	7.50 a	20.80 a	0.71 a
Vetch	**LDPE**	**None**	13.30 a	27.80 a	1.03 a

[w] Plastic films used were as follows: virtually impermeable film (VIF), totally impermeable film (TIF), and low density polyethylene film (LDPE).
[x] All fluorescent *Pseudomonas* data were square root+1 transformed, analyzed, and back-transformed.
[y] Means within the same column followed by the same letter are not significantly different, Waller-Duncan k-ratio *t* test, k=100 or *P*=0.05.
[z] A 79:21 rate of dimethyl disulfide + chloropicrin was used for fumigation.

Table 2.5 *Bacillus, Streptomyces* and fluorescent *Pseudomonas* colony-forming units from soil dilutions, 2013

Treatment				
Cover Crop[u]	**Fumigant**	***Bacillus* spp. CFU X 10^{-5}/g rhizosphere**[v]	***Streptomyces* spp. CFU X 10^{-5}/g rhizosphere**	**Fluorescent *Pseudomonas* CFU X 10^{-3}/g rhizosphere**[w]
Rye	**None**	13.57 a[x]	7.75 a	2.36 b
Rye	**Dimethyl disulfide + chloropicrin**[y]	13.54 a	24.1 a	7.55 ab
Rye	**Methyl bromide + chloropicrin**[z]	17.21 a	10.9 a	1.66 b
Vetch + Rye	**None**	7.54 a	10.6 a	18.98 a
Vetch	**None**	7.42 a	14.9 a	5.05 ab

[u]All treatments used virtually impermeable film (VIF) as the plastic film.

[v,w] *Bacillus* spp. and fluorescent *Pseudomonas* data is only from 3 replications. Data was log transformed, analyzed, and then back-transformed.

[x] Means within the same column followed by the same letter are not significantly different, Waller-Duncan k-ratio *t* test, k=100 or *P*=0.05.

[y] A 79:21 rate of dimethyl disulfide + chloropicrin was used for fumigation.

[z] A 50:50 rate of methyl bromide + chloropicrin was used for fumigation (Terr-O-Gas).

Table 2.6 Average fresh weight biomass and average soil incorporation rate (wt/wt) from cover crops, 2011-2012

Treatment				
Cover Crop	**Plastic Film**[w]	**Fumigant**	**Average Fresh Weight Biomass 2012 (kg/m^2)**	**Average soil incorporation rate (wt/wt)**[x]
Rye	**VIF**	**None**	0.58 a[y]	0.99%
Rye	**VIF**	**Dimethyl disulfide + chloropicrin**[z]	0.60 a	1.01%
Vetch	**VIF**	**None**	0.60 a	0.77%
Vetch	**TIF**	**None**	0.65 a	0.89%
Vetch	**LDPE**	**None**	0.60 a	0.84%

[w] Plastic films used were as follows: virtually impermeable film (VIF), totally impermeable film (TIF), and low density polyethylene film (LDPE).

[x] Wt/wt was calculated by multiplying the dry weight of each cover crop by the area of the plot harvested and dividing it by the weight of the soil in an acre-furrow slice.

[y] Means within the same column followed by the same letter are not significantly different, Waller-Duncan k-ratio *t* test, k=100 or *P*=0.05.

[z] A 79:21 rate of dimethyl disulfide + chloropicrin was used for fumigation.

Table 2.7 Average fresh weight biomass and average soil incorporation rate (wt/wt) from cover crops, 2012-2013

Cover Crop[v]	**Fumigant**	**Average Fresh Weight Biomass 2013 (kg/m^2)**	**Average soil incorporation rate (wt/wt)** [w]
Rye	**None**	0.85 a [x]	1.37%[z]
Rye	**Dimethyl disulfide + chloropicrin**[y]	0.63 ab	1.01%
Rye	**Methyl bromide + chloropicrin** [z]	0.84 a	1.37%
Vetch + Rye	**None**	0.67 ab	1.00%
Vetch	**None**	0.47 b	0.61%

[v]All treatments used virtually impermeable film (VIF) as the plastic film.

[w] Wt/wt was calculated by multiplying the dry weight of each cover crop by the area of the plot harvested and dividing it by the weight of the soil in an acre-furrow slice.

[x] Means within the same column followed by the same letter are not significantly different, Waller-Duncan k-ratio *t* test, k=100 or *P*=0.05.

[y] A 79:21 rate of dimethyl disulfide + chloropicrin was used for fumigation.

[z] A 50:50 rate of methyl bromide + chloropicrin was used for fumigation (Terr-O-Gas).

Table 2.8 Comparison between various cover crops, plastic films, and dimethyl disulfide + chloropicrin and their ammonia concentration at 7 and 20 days post bedding with plastic film, 2012

Treatment				
Cover Crop	**Plastic Film[v]**	**Fumigant**	**Ammonia ppm 7 day**	**Ammonia ppm 20 day**
Rye	**VIF**	**None**	0.09 a[x]	0.08 b
Rye	**VIF**	**Dimethyl disulfide + chloropicrin[y]**	--[z]	0.13 ab
Vetch	**VIF**	**None**	0.68 a	0.17 ab
Vetch	**TIF**	**None**	0.23 a	0.12 ab
Vetch	**LDPE**	**None**	0.20 a	0.47 a

[v] Plastic films used were as follows: virtually impermeable film (VIF), totally impermeable film (TIF), and low density polyethylene film (LDPE).

[w] Ammonia ppm was square root transformed and analyzed before being back-transformed.

[x] Means within the same column followed by the same letter are not significantly different, Waller-Duncan k-ratio *t* test, k=100 or *P*=0.05.

[y] A 79:21 rate of dimethyl disulfide + chloropicrin was used for fumigation.

[z] A 7 day ammonia concentration could not be performed on the fumigated treatment due to label requirements.

Table 2.9 Ammonia concentration (ppm) in soil at 7 days post bedding with virtually impermeable film (VIF), 2013

Cover Crop[v]	**Fumigant**	**Ammonia Concentration 7 day (ppm)**[w]
Rye	**None**	0.11a[x]
Rye	**Dimethyl disulfide + chloropicrin**[y]	--
Rye	**Methyl bromide + chloropicrin**[z]	--
Vetch + Rye	**None**	0.11a
Vetch	**None**	0.57a

[v] All treatments used virtually impermeable film (VIF) as the plastic film.

[w] A 7 day ammonia concentration could not be performed on the fumigated treatments due to label requirements.

[x] Means within the same column followed by the same letter are not significantly different, Waller-Duncan k-ratio *t* test, k=100 or *P*=0.05.

[y] A 79:21 rate of dimethyl disulfide + chloropicrin was used for fumigation.

[z] A 50:50 rate of methyl bromide + chloropicrin was used for fumigation (Terr-O-Gas).

Table 2.10 A mini trial comparison of three plastic films and their ammonia trapping ability, 2012

Treatment		
Plastic Film[y]	**Fertilizer added**	**Ammonia ppm 7 day**
VIF	**Urea**	4.16 a[z]
None	**Urea**	0.60 a
VIF	**None**	0.11 a
LDPE	**Urea**	4.77 a
TIF	**Urea**	3.82 a

[y] Plastic films used were as follows: virtually impermeable film (VIF), totally impermeable film (TIF), and low density polyethylene film (LDPE).

[z] Means within the same column followed by the same letter are not significantly different, Waller-Duncan k-ratio *t* test, k=100 or *P*=0.05.

Table 2.11 Percentage area covered by healthy vines among treatments, 2012

Treatment					
Cover Crop	**Plastic Film[w]**	**Fumigant**	**Percentage Area Covered**	**Percentage Area Covered**	**Change in Percentage Area Covered**
Rye	**VIF**	**None**	74.0 a[y]	58.0 a	-16
Rye	**VIF**	**Dimethyl disulfide + chloropicrin[z]**	77.5 a	47.5 a	-30
Vetch	**VIF**	**None**	84.0 a	66.0 a	-18
Vetch	**TIF**	**None**	77.0 a	73.0 a	-4
Vetch	**LDPE**	**None**	76.0 a	50.5 a	-25.5
Vetch Combined Averages	**--**	**--**	79.0	63.2	-15.8

[w] Plastic films used were as follows: virtually impermeable film (VIF), totally impermeable film (TIF), and low density polyethylene film (LDPE).

[x] Percentage area covered ratings compared the percentage area covered by vines in individual plots to the percentage area covered in the plot with the healthiest and most vigorous vines. Ratings were taken on 8 Jun and 27 Jun 2012.

[y] Means within the same column followed by the same letter are not significantly different, Waller-Duncan k-ratio *t* test, k=100 or *P*=0.05.

[z] A 79:21 rate of dimethyl disulfide + chloropicrin was used for fumigation.

Table 2.12 Percentage area covered by healthy vines among treatments, 2013

Cover Crop[v]	Fumigant	Percentage Area Covered[w]	Percentage Area Covered	Change in Percentage Area Covered
Rye	**None**	65.00 a[x]	68.00 a	3
Rye	**Dimethyl disulfide + chloropicrin[y]**	77.00 a	73.00 a	-4
Rye	**Methyl bromide + chloropicrin[z]**	71.00 a	85.00 a	14
Vetch + Rye	**None**	54.00 a	86.00 a	32
Vetch	**None**	50.00 a	74.00 a	24

[v] All treatments used virtually impermeable film (VIF) as the plastic film.
[w] Percentage area covered ratings compared the percentage area covered by vines in individual plots to the percentage area covered in the plot with the healthiest and most vigorous vines. Ratings were taken on 30 May and 19 Jun 2013.
[x] Means within the same column followed by the same letter are not significantly different, Waller-Duncan k-ratio *t* test, k=100 or *P*=0.05.
[y] A 79:21 rate of dimethyl disulfide + chloropicrin was used for fumigation.
[z] A 50:50 rate of methyl bromide + chloropicrin was used for fumigation (Terr-O-Gas).

Table 2.13 Area under disease progress curve and wilt suppression index, 2012

Treatment				
Cover Crop	**Plastic Film[v]**	**Fumigant**	**AUDPC[w]**	**Wilt Suppression Index[x]**
Rye	**VIF**	**None**	1169 a[y]	0
Rye	**VIF**	**Dimethyl disulfide + chloropicrin[z]**	924 a	6.33
Vetch	**VIF**	**None**	1049 a	9.95
Vetch	**TIF**	**None**	1100 a	12.56
Vetch	**LDPE**	**None**	1292 a	1.66
Vetch Combined Averages	**--**	**--**	--	8.06

[v] Plastic films used were as follows: virtually impermeable film (VIF), totally impermeable film (TIF), and low density polyethylene film (LDPE).

[w] Area under the disease progress curve .

[x] Wilt Suppression Index (WSI) , with the scale of 0 (no suppression) to 100% (total suppression), was calculated by WSI = $(X_{nv}-X_v)/X_{nv}*100$, in which X_{nv} = mean percent wilt in the non-amended control, and X_v = mean percent wilt in the hairy-vetch-amended treatment.

[y] Means within the same column followed by the same letter are not significantly different, Waller-Duncan k-ratio *t* test, k=100 or *P*=0.05.

[z] A 79:21 rate of dimethyl disulfide + chloropicrin was used for fumigation.

Table 2.14 Week 1-9 Percentage of diseased plants based on disease incidence, 2012

Treatment							
Cover Crop	**Plastic Film[x]**	**Fumigant**	**Week 1**	**Week 3**	**Week 5**	**Week 7**	**Week 9**
Rye	**VIF**	**None**	0.25 a[y]	5.65 a	13.08 a	25.56 a	75.24 a
Rye	**VIF**	**Dimethyl disulfide + chloropicrin[z]**	0.00 a	1.43 a	7.64 a	25.56 a	66.16 a
Vetch	**VIF**	**None**	0.00 a	1.21 a	14.45 a	24.69 a	64.26 a
Vetch	**TIF**	**None**	0.00 a	3.95 a	12.41 a	28.22 a	61.36 a
Vetch	**LDPE**	**None**	0.25 a	3.21 a	11.76 a	36.63 a	73.50 a

[x] Plastic films used were as follows: virtually impermeable film (VIF), totally impermeable film (TIF), and low density polyethylene film (LDPE).

[y] All rating data were transformed using arc-sine square root analyzed, and then back-transformed as shown. Means within the same column followed by the same letter are not significantly different, Waller-Duncan k-ratio *t* test, k=100 or *P*=0.05.

[z] A 79:21 rate of dimethyl disulfide + chloropicrin was used for fumigation.

Table 2.15 Week 3-9 Percentage of diseased plants based on disease incidence, area under the disease progress curve, and wilt suppression index, 2013

Cover Crop[u]	Fumigant	Week 3	Week 5	Week 7	Week 9	AUDPC[v]	Wilt Suppression Index[w]
Rye	**None**	1.95 a	6.61 a	8.18 a	15.89 a	419 a	0.00
Rye	**Dimethyl disulfide + chloropicrin[y]**	4.76 a	6.61 a	6.61 a	12.41 a	460 a	13.00
Rye	**Methyl bromide + chloropicrin[z]**	3.57 a	6.61 a	8.73 a	8.73 a	548 a	27.00
Vetch + Rye	**None**	4.76 a	8.18 a	13.08 a	18.92 a	528 a	0.00
Vetch	**None**	2.86 a	7.64 a	12.41 a	18.14 a	545 a	0.00

[u] All treatments used virtually impermeable film (VIF) as the plastic film.

[v] Area under the disease progress curve.

[w] Wilt Suppression Index (WSI) , with the scale of 0 (no suppression) to 100% (total suppression), was calculated by WSI = $(X_{nv}-X_v)/X_{nv}*100$, in which X_{nv} = mean percent wilt in the non-amended control, and X_v = mean percent wilt in the hairy-vetch-amended treatment.

[x] All rating data were transformed using arc-sine square root analyzed, and then back-transformed as shown. Means within the same column followed by the same letter are not significantly different, Waller-Duncan k-ratio *t* test, k=100 or *P*=0.05.

[y] A 79:21 rate of dimethyl disulfide + chloropicrin was used for fumigation.

[z] A 50:50 rate of methyl bromide + chloropicrin was used for fumigation (Terr-O-Gas).

Figure 2.1 Disease progress curve from cover crop study, 2012

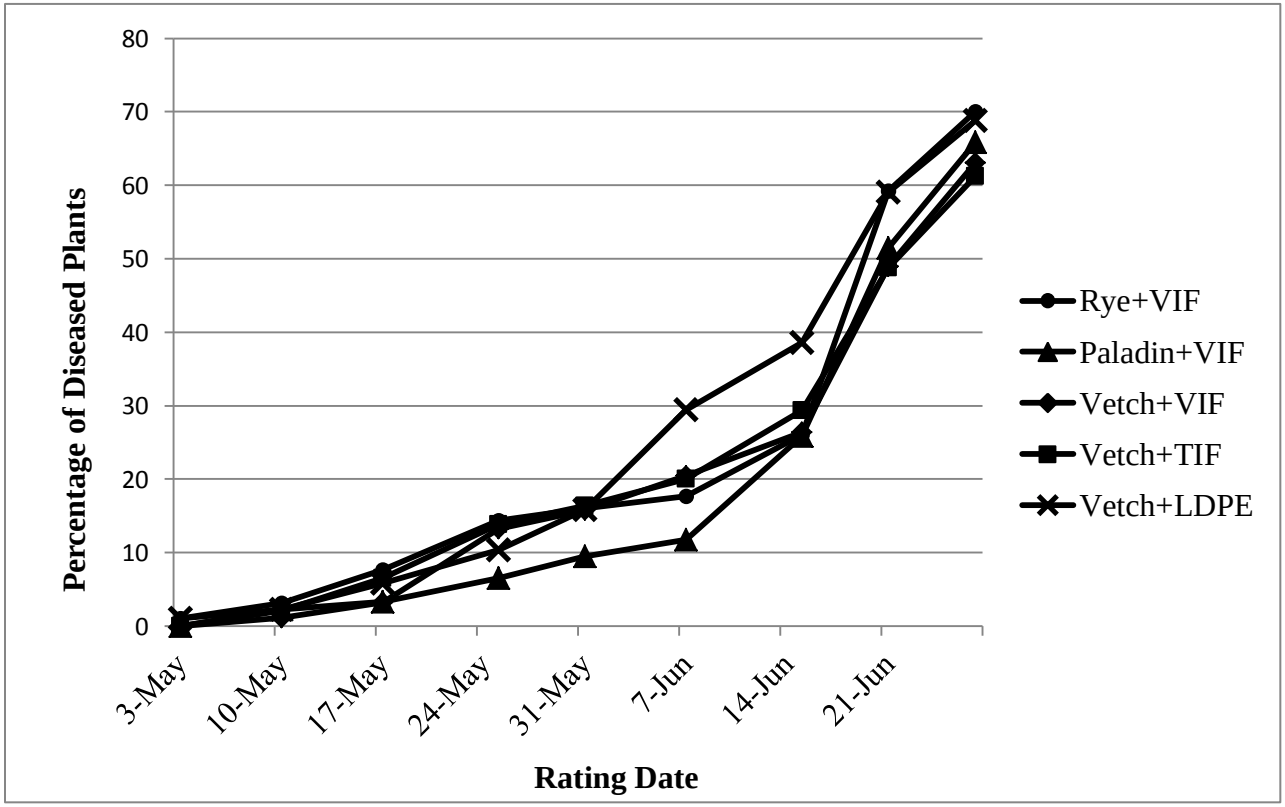

Figure 2.2 Disease progress curve from cover crop study, 2013

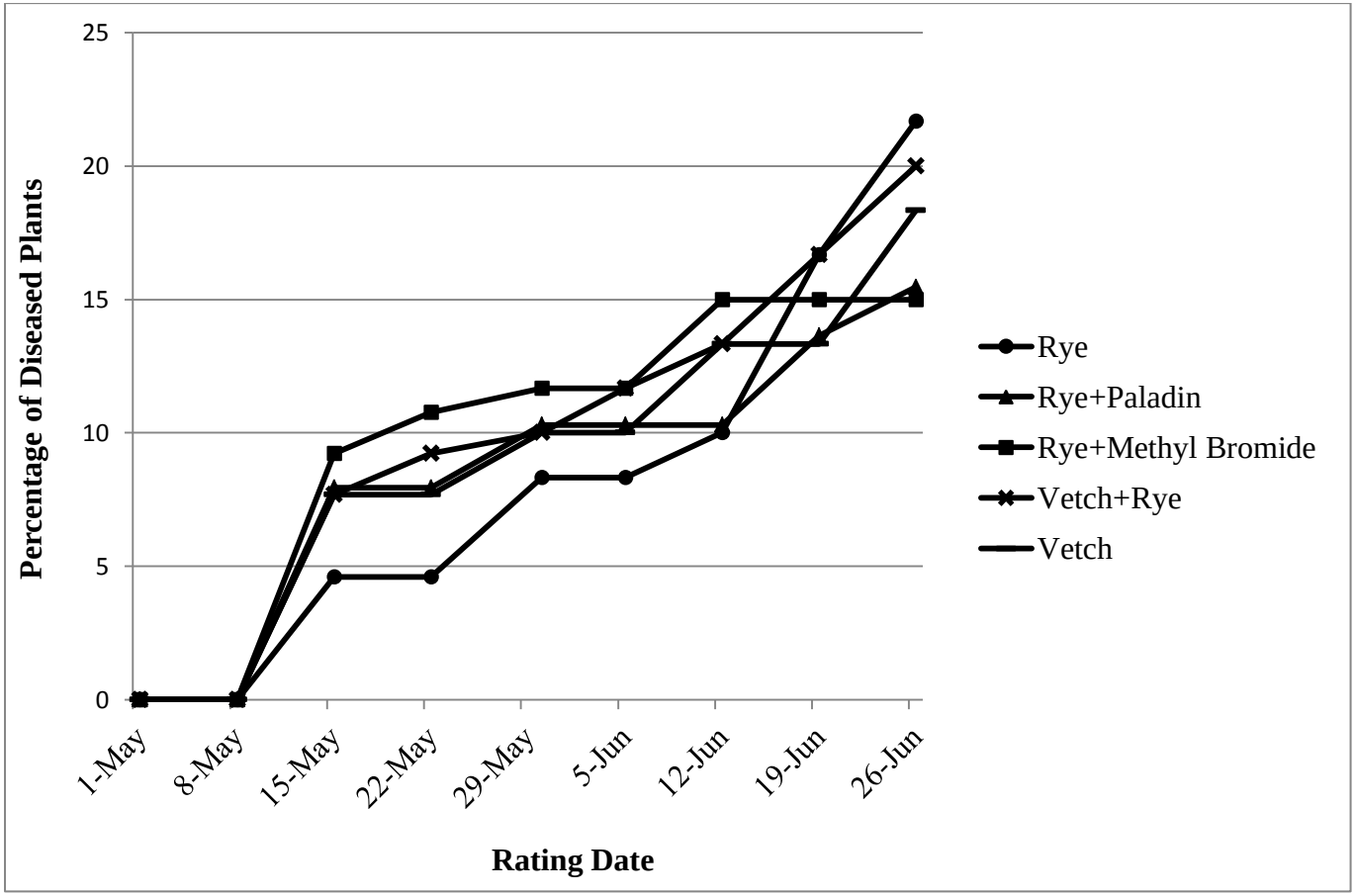

Table 2.16 Average total marketable yield and average number of marketable watermelons, 2012

Treatment					
Cover Crop	**Plastic Film[x]**	**Fumigant**	**Average Marketable Yield (kg/21.34m)**	**Average Marketable Yield (kg/ha)**	**Average Number of Marketable Watermelons per Treatment**
Rye	**VIF**	**None**	110.56 a[y]	14155 a	20.6 a
Rye	**VIF**	**Dimethyl disulfide + chloropicrin[z]**	118.89 a	15222 a	22.0 a
Vetch	**VIF**	**None**	114.64 a	14678 a	23.8 a
Vetch	**TIF**	**None**	132.31 a	16940 a	25.2 a
Vetch	**LDPE**	**None**	106.72 a	13664 a	20.0 a

[x] Plastic films used were as follows: virtually impermeable film (VIF), totally impermeable film (TIF), and low density polyethylene film (LDPE).

[y] Means within the same column followed by the same letter are not significantly different, Waller-Duncan k-ratio *t* test, k=100 or *P*=0.05.

[z] A 79:21 rate of dimethyl disulfide + chloropicrin was used for fumigation.

Table 2.17 Average marketable yield and average number of marketable watermelons, 2013

Cover Crop[w]	Fumigant	Average Marketable Yield (kg/15.24m)	Average Marketable Yield (kg/ha)	Average Number of Marketable Watermelon per Treatment
Rye	**None**	112.44 ab[x]	20159 ab	20.6 ab
Rye	**Dimethyl disulfide + chloropicrin[y]**	143.65 ab	25753 ab	19.8 ab
Rye	**Methyl bromide + chloropicrin[z]**	167.58 a	30044 a	24.4 a
Vetch + Rye	**None**	135.27 ab	24252 ab	19 ab
Vetch	**None**	102.61 b	18395 b	16.2 b

[w] All treatments used virtually impermeable film (VIF) as the plastic film.
[x] Means within the same column followed by the same letter are not significantly different, Waller-Duncan k-ratio *t* test, k=100 or *P*=0.05.
[y] A 79:21 rate of dimethyl disulfide + chloropicrin was used for fumigation.
[z] A 50:50 rate of methyl bromide + chloropicrin was used for fumigation (Terr-O-Gas).

Table 2.18 Root and stem tissue sampling for *Fusarium oxysporum* and *Pythium* throughout the 2012 season

	Treatment			Roots		Stem
Date Sampled	**Cover Crop**	**Plastic Film[v]**	**Fumigant**	***Fusarium oxysporum*[w]**	***Pythium***	***Fusarium oxysporum***
5/25/12	**Rye**	**VIF**	**None**	1/4[x]	2/4	0/4
	Rye	**VIF**	**Dimethyl disulfide + chloropicrin[y]**	0/4	1/4	1/4
	Vetch	**VIF**	**None**	0/4	0/4	0/4
	Vetch	**TIF**	**None**	1/4	1/4	4/4
	Vetch	**LDPE**	**None**	1/4	1/4	4/4
6/22/12	**Vetch**	**LDPE**	**None**	2/8	8/8	8/8
	Rye	**VIF**	**Dimethyl disulfide + chloropicrin**	--	--	8/8
	Vetch	**TIF**	**None**	--	--	4/4
7/16/12	**Rye**	**VIF**	**None**	4/4	--	4/4
	Rye	**VIF**	**Dimethyl disulfide + chloropicrin**	2/2	--	2/2
	Vetch	**VIF**	**None**	2/2	--	2/2
	Vetch	**TIF**	**None**	2/2	--	2/2
	Vetch	**LDPE**	**None**	2/2	--	2/2

[v] Plastic films used were as follows: virtually impermeable film (VIF), totally impermeable film (TIF), and low density polyethylene film (LDPE).
[w] The May 25, 2012 and June 22, 2012 samples were from plants that showed non-typical symptoms of Fusarium wilt.
[x] Each row refers to one or two plants; numbers correspond to the number of pieces with the pathogen present.
[y] A 79:21 rate of dimethyl disulfide + chloropicrin was used for fumigation.
[z] The 16 July 2012 samples were all from plants that had collapsed and were on the verge of dying.

Table 2.19Root and stem tissue sampling for *Fusarium oxysporum* and *Pythium* throughout the 2013 season

			Roots		Stem
Date Sampled	**Cover Crop**	**Fumigant**	***Fusarium oxysporum***[v]	***Pythium***	***Fusarium oxysporum***
5/8/13	**Vetch**	**None**	2/4[w]	2/4	3/4
5/15/13	**Vetch + Rye**	**None**	1/8	8/8	--[x]
	Rye	**Methyl bromide + chloropicrin**[y]	1/4	4/4	--
	Rye	**Dimethyl disulfide + chloropicrin**[z]	1/4	4/4	--
	Vetch	**None**	1/4	4/4	--
	Rye	**None**	1/2	2/2	--
5/22/13	**Vetch + Rye**	**None**	--	--	2/4
6/19/22	**Rye**	**Dimethyl disulfide + chloropicrin**	4/4	0/4	4/4
	Rye	**None**	--	--	8/8
	Vetch	**None**	--	--	4/4
	Rye	**Methyl bromide + chloropicrin**	--	--	8/8

[v] Plants sampled showed non-typical symptoms of Fusarium wilt.
[w] Each row refers to one or two plants; numbers correspond to the number of pieces with the pathogen present.
[x] -- means that no samples were cultured.
[y] A 50:50 rate of methyl bromide + chloropicrin was used for fumigation (Terr-O-Gas).
[z] A 79:21 rate of dimethyl disulfide + chloropicrin was used for fumigation.

CHAPTER THREE

GRAFTING STUDY

Introduction

Grafting vegetables is a practice that was first used to manage Fusarium wilt in watermelon (King *et al.*, 2008). Grafting has become an increasingly popular technique explored to manage soilborne pests since the banning of methyl bromide began. Grafting has been used for decades in the Middle East and in Asia but has not been a technique adopted by the western world until recently (Cohen *et al.*, 2007). Its lack of use can be attributed to many factors including methyl bromide critical use exemptions, higher initial investment, perceived unverified technology, and more extensive farming systems (King *et al.*, 2008). With the phase-out of methyl bromide more focus has shifted away from fumigants and more attention has been given towards alternative methods including grafting (Cohen *et al.*, 2007) . Having rootstocks that are resistant to disease as well as proficient grafting techniques, grafting has expanded into a worldwide practice. Grafting provides many benefits that could only be achieved by the conjoining of two plants. The rootstocks used in vegetable grafting have been specifically chosen from wild genotypes that exhibit desirable qualities such as specific disease resistance. Rootstocks also provide a more developed and vigorous root system when compared to non-grafted watermelons (Guan *et al.*, 2012). With a stronger root system, plants are less affected by pathogens thus water and nutrient uptake is not compromised. Higher concentrations of nitrates have been found in grafted plants when compared to self-rooted melons. More vigorous

growth of the root system and conversely the above ground plant leads to healthier plants that have a higher disease tolerance than non-grafted plants. Augmented uptake of nutrients also plays a major role in plant defense pathways as these essential nutrients are used to elicit defense responses within the plant. It has been shown that plants that are grafted onto a resistant rootstock can exhibit higher levels of genes that are related to stress than plants that are grafted onto susceptible rootstocks thus providing better overall disease protection (Guan *et al.*, 2012).

Rhizosphere microbial communities are very important when studying soilborne diseases. There are many microbes such as beneficial bacteria and actinobacteria that can offer protection from soilborne pathogens. In a study where cucumber was grafted onto *C. moschata* rootstock the population of beneficial bacteria and actinobacteria was increased in the rhizosphere while the number of fungi was reduced (Guan *et al.*, 2012). The same trend was seen in grafted peppers and eggplant. The increased ratio of beneficial bacteria and actinomycetes to fungi in these grafted vegetables may be aiding in the enhanced disease protection that the rootstock can offer (Guan *et al.*, 2012).

Rootstock Selection

Rootstock selection is a critical factor when grafting due to the fact that a decision has to be made as to what is the purpose of the grafting. One of the most intriguing aspects to grafting is increased fruit size which corresponds to higher yields. Along with increasing yields, grafting can also increase desired traits such as fruit quality (Louws *et al.*, 2010). Grafting can be used for disease protection, however if there is no

disease pressure then a rootstock may be selected for another reason. Grafting can allow a grower to plant earlier in the season due to a rootstock's ability to tolerate lower soil temperatures. Also grafting may allow plants to be grown in sub-optimal soil conditions such as saline soils (Yetisir *et al.*, 2003). In some cases intraspecific grafting, or grafting onto the same species, is acceptable, while in other instances interspecific grafting, where grafting involves a member of a closely related botanical family, is needed. Many times intraspecific grafting is used to manage specific pests in which the rootstock may have resistance genes that the scion does not have, but the desired fruit quality lies within the scion. Interspecific grafting provides broad and nonspecific protection from a wide array of soilborne pathogens (Cohen *et al.*, 2007).

In recent studies to manage Fusarium wilt, interspecific grafting methods have been explored. *Lagenaria* rootstocks have shown higher survival rates in watermelon grafting when compared to the *Cucurbita* rootstocks due to incompatibility issues not seen in *Lagenaria* rootstocks (Yetisir *et al.*, 2003). This may be attributed to watermelons being more closely related to *Lagenaria* rootstocks than hybrid squash rootstocks. Watermelon and bottle gourds are in the same tribe as well as the same subtribe. Watermelon and squash are in the same subfamily but are not in the same tribe or subtribe (Robinson & Decker-Walters, 1997). In screenings with a very susceptible scion, Sugar Pack, it was shown that both Strong Toza (assumed to be Strong Tosa) and Emphasis reduced crown and root rot as well as Fusarium wilt when compared to susceptible commercial non-grafted cultivars (Boughalleb *et al.*, 2007). In work by Keinath and Hassell both Strong Tosa (*Cucurbita* rootstock) and Emphasis (*Lagenaria*

rootstock) had significant Fusarium wilt reduction when compared to the susceptible Tri-X 313 and the Tri-X 313 self-graft (Keinath & Hassell, 2014).

Fusarium oxysporum f. sp. *lagenariae*

While grafting is a beneficial option in an integrated approach to managing Fusarium wilt, it too has its limitations. The forma specialis *lagenariae* of *Fusarium oxysporum* has been found to be pathogenic on bottle gourds as well as pumpkin and Malabar gourds (*Cucurbita ficifolia*) (Namiki *et al.*, 1994). When watermelons were grafted onto bottle gourd rootstocks, they wilted suddenly even in the presence of low inoculum levels of *Fusarium oxysporum* f. sp. *lagenariae*. Interestingly enough, bottle gourds that were not grafted showed no wilt even under the same inoculum levels which confirms a complex interaction because they were grafted (Louws *et al.*, 2010). Due to their ability to infect a few of the same species of cucurbits, the genetic relationships between *niveum*, *lagenariae*, and other forma specialis has been investigated by researchers. If two formae speciales share the same host, then it is possible that the two are genetically related and contain traits that are needed to be pathogenic (Namiki *et al.*, 1994). In the 1950's the rootstock that was used to manage Fusarium wilt in Japan began experiencing wilt symptoms. It was discovered that *Fusarium oxysporum* f. sp. *lagenariae* was the causal organism responsible for the disease. Breeding programs were developed to overcome the susceptibility or the rootstocks to *Fusarium oxysporum* f. sp. *lagenariae*. It is proposed in the future, due to selection pressure that the pathogen will evolve and become pathogenic on improved rootstocks. However rootstocks, like crops,

can be rotated to avoid selection pressure. Varying cultivars from the same species as well as rootstocks from different species can be rotated to maintain resistance across all rootstocks (King *et al.*, 2008).

Objectives

The objective of this study was to evaluate the efficacy of grafting, race-1 resistance, and fumigation on Fusarium wilt management in watermelon production. Disease progression, percentage area covered by healthy vines, and yield were all used as to evaluate each treatment's impact on disease management. Pathogen isolations and pathogenicity testing were both used to confirm the causal agents of disease in the study.

Materials and Methods

The experiment was conducted in a field of Yonges loamy fine soil at Clemson University's Coastal Research and Education Center, Charleston, South Carolina. *Fusarium oxysporum* f.sp. *niveum* (FON) populations had been previously documented in the field. Additionally, a watermelon cultivar susceptible to Fusarium wilt was planted in the field in the summer of 2011 and 2012. In both field seasons, the design of the experiment was a Latin Square with six treatments and six replications per treatment. The treatments consisted of Tri-X 313 watermelons grafted onto three rootstocks; Emphasis, Strong Tosa, and Tri-X 313, watermelon cultivar 'Fascination', resistant to FON race 1; non-grafted Tri-X 313 with Paladin, a dimethyl disulfide + chloropicrin fumigant; and a non-grafted, non-fumigated control treatment. The field was seeded to

rye (67 kg/ha) on 27 October 2011 and 18 October 2012. The rye was mowed and disked on 7 March 2012 and 14 January 2013. Fertilizer (15-0-15) was added at a rate of 560 kg/ha on 21 March 2012 and disked in on 22 Mar 2012. The same fertilizer application was repeated on 20 March 2013. Dual Magnum (1555 ml/ha) and Ridomil Gold (2338 ml/ha) were sprayed before beds were pressed and covered with virtually impermeable film (VIF) on 29 March 2012. Sandea (73 ml/ha) was sprayed on 20 Mar 2013 before beds were pressed and covered with VIF. Dimethyl disulfide + cholorpicrin (467.69 L/ha) was applied on 29 March 2012 and 21 March 2013.

All plants were transplanted on 19 April 2012 and were spaced 1.22 meters apart within rows with pollenizers (SP-5) transplanted in-between every fourth plant in 2012. Each plot was 15.24 m long with 0.9-meter-wide beds on 1.83-meter-centers in 2012 and 14.33 m long with the same bed dimensions in 2013. Transplants were planted on 16 April 2013 with the same procedure as 2012 with the only exception being the use of 'SP6' pollenizers. In the 2012 season the 'SP5' pollenizers performed poorly, so a new pollenizer, 'SP6,' was used in 2013. Insects, foliar pathogens, and weeds were managed in the grafting study in the same manner as those in the cover crop study.

Grafting Procedure

Tri-X 313 and 'Fascination' watermelon were seeded in the greenhouse on 20 February 2012 and 28 February 2013. Emphasis and Strong Tosa rootstocks were seeded on 24 and 27 February 2012, respectively. Emphasis rootstock was seeded on 4 March 2013 while Strong Tosa and 'SP6' pollenizers were seeded on 6 March 2013. Offsetting

the planting dates of the rootstock and the scion ensured that the diameter of the stem where they were grafted was very similar. On 19 March 2012 and 25 March 2013, Tri-X 313 scions were grafted onto Emphasis, Strong Tosa, and Tri-X 313 rootstocks using the single-cotyledon grafting method. The single cotyledon grafting method involved cutting a 45 degree angle in which one cotyledon of the rootstock was removed. The scion was also cut at a 45 degree angle and the two plants were joined. A small plastic clip was attached at the junction of the graft to hold the rootstock and scion together while healing. The plants were placed into a grafting chamber with high humidity and low light for 4 to 7 days (Kubota *et al.*, 2008). After healing in the humidity chamber, the plants were allowed to acclimate in the greenhouse for a few days before being transplanted into the field on 19 April 2012 and 16 April 2013.

Disease Progression and Harvest

Watermelon transplants were monitored once a week for disease symptoms starting on 3 May 2012, two weeks post-transplant, and continued until 27 Jun 2012. In the 2013 study scouting for Fusarium wilt began on 1 May 2013 and continued until 26 June 2013. The procedure for monitoring disease was performed in both field experiments as described in the cover crop study. In 2013, a percentage area covered rating was taken in addition to disease incidence ratings. This rating takes the percentage area covered of the healthiest plot and compares it to the other plots. A correlation between percentage area covered and disease severity was made between the rye control and the dimethyl disulfide + chloropicrin treatments because the same cultivar was used.

Marketable and wildlife damaged watermelons were harvested when ripe and weighed once a week for four weeks on 2, 9, 16, and 23 July 2012 and 28 June 3, 10, and 17 July 2013 to obtain a total marketable yield for the field season. Ripened small, sunburned, and deformed watermelons were not measured as part of the study.

Pythium and *Fusarium* Sampling

In addition to checking plants weekly for disease, the stem and roots of plants grafted onto Emphasis rootstock that were severely stunted were collected on 25 May and 12 June 2012. Previously marked diseased Emphasis plants that appeared to be healthy on 2 July 2012 were selected for *Pythium* and *Fusarium* testing as well. These plants were marked as diseased on the second rating week but had appeared to recover and become seemingly healthy. The stems of the rootstock and scion of diseased plants were cross sectioned with a razor blade a few centimeters above the roots and graft union, respectively. The cross sectioned stems were surface-disinfested in a 0.6% sodium hypochlorite solution for 1.5 minutes. The stems were rinsed in sterilized distilled water for 30 seconds and then blotted until dry. The stems were cut into four 0.2 x 0.1 cm sections, containing the vascular bundles, and placed on Komada's *Fusarium*-selective medium and left in the light to incubate for 5 days. Colonies of *Fusarium oxysporum* were identified based on formation of salmon-colored colonies (Zhou & Everts, 2003). Small pieces, 0.5 cm to 1.0 cm, of discolored root tissue were cut from the diseased plants. The diseased roots were surface disinfested in a 0.3% sodium hypochlorite solution for 30 seconds, rinsed, and blotted. Roots were placed onto water agar +

streptomycin (100 mg/l) medium and allowed to incubate in the light for 1-3 days. *Pythium* and *Fusarium* colonies were observed under a dissecting microscope and recorded.

Pathogen Isolation

Isolates were prepared and saved for pathogenicity testing from the 12 June 2012 sampling of severely diseased Emphasis plants. A small piece of agar containing *Fusarium* mycelia from one colony of the stem isolation was cut with a sterilized scalpel and placed on quarter strength potato dextrose agar (PDA). This process was done so that there were two pieces of agar and mycelium on one quarter strength PDA plate. The quarter strength PDA plates were allowed to incubate for 3 to 4 days until colonies began to sporulate. A small piece of agar with spores was chosen from one colony on the quarter strength PDA plate. The small piece of agar with spores was added to a test tube containing 3 ml of sterilized water and vortexed for 30 seconds. 200 µl of the vortexed suspension was added to water + streptomycin (WAS) agar plate and spread using a "Y"-shaped glass rod. Twenty-four hours later, germinating single spores were isolated using a dissecting microscope and cut out of the agar using a sterile scalpel and added to quarter strength PDA plates. After colonies of *Fusarium* had formed, a small piece of agar and mycelia were removed and added to a quarter strength PDA plate that contained one piece of sterilized filter paper. Two pieces of agar with mycelia were added on opposite ends of the quarter strength PDA plate with filter paper. After 3 weeks the filter paper was removed from the agar and added to a sterilized petri dish. The filter paper

was allowed to dry for several days. Once dry, the filter paper with *F. oxysporum* spores was cut into tiny pieces and added to a 20 ml vial. Isolates from Emphasis plants were named ZEG and isolates from 'SP5' and 'SP6' plants were named 'SP5' and 'SP6.'

Quick Pathogenicity Testing

When isolates were screened for pathogenicity, a quick pathogenicity test was performed on either Black Diamond or Sugar Baby to see if the isolates that were being tested were in fact pathogenic on watermelon (Zhou & Everts, 2003). These two cultivars are highly susceptible to the Fusarium wilt pathogen. The next step, if isolates were deemed pathogenic, was to perform a series of pathogenicity tests on a set of watermelon differentials that determined the race of FON. (Kleczewski & Egel, 2011)

Each isolate was grown on quarter strength PDA from the filter paper saved from the 20 ml vial. Two pieces of filter paper were added to the agar and allowed to grow for 5-8 days under room temperature and 16 hour days under fluorescent light. A 4 mm core from each culture was added to 10 ml of a sterile mineral salts broth in a 20 ml vial. Each of the cultures was strapped to a shaker table at 135 rpm for six days. In addition to each culture, a negative control (sterile mineral salts broth), as well as a control for race 1 and 2 FON, had to be prepared. Fafard 3B potting soil was filled 2/3 full in 72 cell flats. Three seeds were placed in each cell and replicated twice in a randomized complete block design. Macis (*Lagenaria siceraria*) and Sugar Baby (*Citrullus lanatus*) were used as the two hosts. After six days on the shaker table spores of the isolates had begun to form. Each vial was gently shaken to homogenize the spores. One and one half ml of each

culture was added to each cell with the previously sown seeds. A layer of potting soil was added on top of the seeds before being lightly watered and left in the greenhouse. The plants were checked once a week for three weeks for disease symptoms. If one plant from each isolate had Fusarium symptoms, then that isolate was marked as pathogenic and was used later for race testing.

In a quick pathogenicity test on 'SP6' isolates, germinated seeds were inoculated, 72 hours post-seeding, with inoculum. Because spore counts were low in some isolates, the broth containing the isolates was blended for ten seconds to grind up both spores and mycelium. The inoculum was added to the germinated seedlings and lightly watered in. Seeds were allowed to germinate to ensure that the seeds had germinated and that the symptoms were due to Fusarium wilt and not poor germination. In addition to the mineral salts broth control and race 1 & 2 controls, water, and a non-pathogenic *F. oxysporum* were also used as controls.

The same procedure for determining the ratio of pathogenic to nonpathogenic *Fusarium oxysporum* was performed in the rye control and the dimethyl disulfide + chloropicrin treatments.

Race Determination of FON

Fifteen 72-cell flats were filled with Fafard 3B potting mix on 23 Jan 2013. Five of the flats were seeded with Sugar Baby, five with Crimson Sweet, and the remaining five with Allsweet. These three cultivars served as a set of differentials to determine the race of isolates of FON (Zhou & Everts, 2003). Flats were covered with soil, watered,

and placed on a heating pad. Additionally, a trash bag was cut open and used to cover the flats to create a humid environment that would promote germination. After two days the trash bags used to cover the flats were removed. Flats were left in the greenhouse for two weeks. Two liters of potato dextrose broth was made and inoculated with all field isolates as well as a race 2 control isolate. A control race 0 and race 1were used in this pathogenicity testing.

Flasks filled with 100 ml of broth and three 1-cm pieces of agar containing each of the isolates were placed on a shaker table for 7 days at 125 revolutions per minute (rpm) to allow spores to develop. After seven days of spore production, flasks were taken off the shaker table. The cultures were then poured through two layers of cheesecloth into a beaker to filter spores from chunks of mycelium. The spore solution was added to centrifuge tubes and spun at 4500 rpm for five minutes. The supernatant was poured off and the remaining pellet was vortexed to re-suspend the spores. 100 µl of the solution was added to 9.9 ml of water and vortexed. A spore count was taken using a hemocytometer and each isolate suspension was adjusted to reach 10^6 conidia to achieve a total volume of 200 ml. Each suspension was poured into a 6.5 cm X 6.5 cm square box. Roots of twenty plants from each cultivar were washed free of soil. The roots were dipped into the spore suspension by cultivar for a minimum of 30 seconds. Four plants from one cultivar were planted into one pot and replicated five times. This same procedure was repeated for all three cultivars of watermelon for each isolate. The experimental design was a randomized complete block design with five replications. Plants were put back into the greenhouse to allow disease symptoms to develop once a

week for three weeks (Zhou & Everts, 2004). For an isolate to be considered race 0, greater than 15% wilt must occur on Sugar Baby and a mean wilt incidence of less than 33% must occur on all other cultivars. If ≤ 50% wilt occurs on Calhoun Gray, Dixielee, and Allsweet and an average wilt incidence between 33 and 66% on all other cultivars exists then an isolate is noted as race 1. If greater than 50% wilt occurs on Allsweet seedlings and an average wilt incidence higher than 66% on all other cultivars occurs then an isolate is considered race 2 (Zhou & Everts, 2003). In this study Crimson Sweet was used as a race 1 indicator due to better germination rates than other race 1 indicators.

On 8 February 2013, Sugar Baby, Crimson Sweet, Allsweet, and Charleston Gray seeds were sown to repeat the pathogenicity testing. The procedure was repeated as mentioned as above with the addition of Charleston Gray watermelon and race 0 and 1 control isolates. Only Emphasis (ZEG) and control isolates were used in the testing. The test was not performed after the root dip due to excessive wilting immediately after transplanting. The root systems on the watermelons were less extensive than normal and the roots themselves may have been washed too vigorously before dipping in the spore suspension thus causing irreversible recovery.

Pathogenicity Testing for *Fusarium oxysporum* f. sp. *lagenariae*

On 20 February 2013 four-98 cell flats of five different Cucurbit cultivars were seeded for pathogenicity test 5. Cultivars used were as follows: Emphasis (*Lagenaria siceraria*), Connecticut Field Pumpkin (*Cucurbita pepo*), Dill's Atlantic Giant (*Cucurbita maxima*), Bottlegourd (*Lagenaria siceraria*), and Waltham Butternut (*Cucurbita*

moschata). Only Emphasis (ZEG) isolates and control isolates were tested. Fifteen days after germination, at the first true leaf stage, roots were washed and root dipped in a spore suspension as described previously. Each isolate was replicated five times, each containing three plants in a replication, with five different cultivars. Plants were rated at seven and fourteen days after the roots had been dipped in the spore suspension. After the fourteen day replication, plants were discarded as no disease symptoms had developed. A total of seven pathogenicity tests were performed to identify pathogenic isolates of *F. oxysporum* (see Table 3.10).

Statistical Analysis

All data was analyzed using SAS software package (version 9.2 and 9.3; SAS Institute Inc., Cary, NC). Analysis of variance was performed using PROC MIXED or PROC GLM on data and means were compared using the Shapiro-Wilke least significant difference at $P = 0.05$. Ammonia concentrations were square root transformed before being analyzed due to non-normally distributed data in 2012. Bacteria data were transformed using the log or log + 1 transformation. Prior to analysis, disease incidence data was transformed using the arcsine square root transformation. Contrast-analyses were performed to examine any differences among groups of treatments. Additionally, a repeated measures analysis was used to examine differences in CFUs over time. Area under the disease progress curve was calculated by the sum PROC means and then analyzed by PROC GLM.

Results

Colony-forming units (CFU)

Soil was sampled at two depths, 0-10 cm and 10-20 cm, in April and May 2013 to evaluate the effect of dimethyl disulfide + chloropicrin on CFUs of *F. oxysporum* when compared to a non-fumigated control (Table 3.1). At both sampling dates and sampling depths, and when data from the two depths were combined, there were significantly lower CFUs in the fumigated treatment than in the non-fumigated rye treatment. There was no change in CFUs in the fumigated treatment from the April to the May sampling but there was a significant decrease in CFUs in the control treatment during the same time period.

Disease incidence, AUDPC, percentage area covered and wilt suppression index

In 2012 there were no differences in disease incidence among treatments in any rating week. Although statistically similar to other treatments, Strong Tosa and Emphasis had the least amount of disease (12%), followed by 'Fascination' with 20% disease incidence (disease percentage is raw data, not the back-transformed data presented in Table 3.2). The dimethyl disulfide + chloropicrin treatment had 32% disease incidence, which was more than any other treatment. When grouped together in a contrast-analysis, disease incidence of Emphasis and Strong Tosa was not significantly different from the non-grafted control (P=0.10).

In 2013, the self-grafted Tri-X 313 and Tri-X 313 non-grafted treatments had the highest disease incidence of all treatments (Table 3.3). In contrast-analysis, Emphasis, Strong Tosa, 'Fascination', and dimethyl disulfide + chloropicrin treatments were grouped together and had a significantly lower disease incidence compared to the controls (self-graft and non-grafted) (P=0.002). When grouped together and compared to the two controls (self-grafted and non-grafted), Emphasis and Strong Tosa also had significantly lower disease incidence (P=0.0012). Additionally, dimethyl disulfide + chloropicrin was significantly lower than the two controls (P=0.032) but was not significantly different from Emphasis and Strong Tosa grouped together (P=0.44). The 'Fascination' treatment did not significantly lower disease incidence when compared to the two controls in 2013 (P=0.13).

With the exception of the Tri-X 313 non-grafted treatment in 2013, the disease progression followed a similar pattern in both years with all treatments (Figures 3.1 & 3.2). Disease was first observed in the second rating week, three weeks post-transplant, and then plateaued for a few weeks where no additional plants with symptoms were found. By the seventh rating week when fruit size was increasing, there was an increase in disease until the last rating week. The Tri-X 313 self-graft treatment had an 8% disease incidence in week 7 but increased to over 25% by the final rating week in 2013; this was the highest increase in disease incidence over the two-week span. The Tri-X 313 non-grafted treatment had a steady increase in disease throughout the 2013 growing season without experiencing the plateau period seen in other treatments.

There were no differences in the AUDPC among any treatments in 2012 or 2013 (Tables 3.2 & 3.3). Strong Tosa, the least diseased treatment in 2012, had a value of 325 for AUDPC. In 2013, Emphasis had the lowest AUDPC at 44, while Strong Tosa had the second lowest at 129. In 2013, percentage area covered was rated to assess the health of the plants in each plot. The Emphasis and the self-grafted treatments were the only treatments to have a lower rating at the second rating than at the first rating (Table 3.4). The Tri-X 313 non-grafted treatment had no change in rating while the percentage area covered in the Strong Tosa, 'Fascination', and fumigated treatments increased from the first to the second rating. In the first rating, Strong Tosa, which had the lowest percentage, had a statistically lower rating compared to the Emphasis and self-grafted treatments. A percentage area covered rating was not taken in 2012.

Strong Tosa had the highest wilt suppression index value in both years, reducing wilt compared to the control by 50% in 2012 and by 80.91% in 2013 (Tables 3.2 & 3.3). Emphasis had a 49.49% wilt suppression index in 2012 and 71.58% in 2013. The fumigated treatment had a 0% WSI in 2012 and 45.22% in 2013. 'Fascination' had a relatively consistent wilt suppression index with 21.72% and 36.93% in 2012 and 2013, respectively.

Watermelon harvest

In both 2012 and 2013, there were no significant differences in yield among any of the treatments (Tables 3.5 & 3.6). The marketable yields were statistically higher in 2012 than in 2013, although there was no effect of treatment or treatment-by-year

interaction. The marketable yield in the lowest treatment in 2012 was similar to the highest treatment in 2013. The total number of marketable watermelons was the same (33.5) in both the Emphasis and self-grafted treatments in 2012. The Tri-X 313 self-grafted treatment in 2012 had the highest yield of any treatment in any year with 42,895 kg/ha of marketable watermelons.

Sampling for *Fusarium oxysporum* and *Pythium* in diseased plants

In 2012, 30% of Emphasis root pieces that were cultured from the first two samplings were found to have *F. oxysporum* and 50% were found to have *Pythium.* It was also found that 90% of the stem pieces from the rootstock had *F. oxysporum* and 60% of the scion contained *F. oxysporum* in the same plants (Table 3.7). From the diseased plant sampling at the end of the season, which included diseased plants from all treatments, 19% of root pieces had *Pythium* while 86% of the stem or vine samples contained *F. oxysporum* (Table 3.7). In 2013, 70% of root pieces sampled from the entire growing season contained *Pythium* while only 11% had *F. oxysporum* (Table 3.9). Fifty percent of stem pieces from non-grafted plants contained *F. oxysporum.* In the grafted plants, 70% of the pieces from the rootstock and 20% from the scion had *F. oxysporum* infection.

In 2012, one healthy and one diseased plant from each treatment were sampled and two pieces from both the rootstock and scion were cultured on Komada's medium. At least 67% of the pieces sampled in each treatment contained *F. oxysporum* (Table

3.8). There were no differences between healthy and diseased plants or between rootstock and scion material in the presence of *F. oxysporum*

From the isolates collected from both the rootstock and scion of diseased Emphasis grafted plants in 2012, none were pathogenic forms of *F. oxysporum* (Table 3.10). In addition, none of the 'SP5' pollenizer isolates were FON. From initial quick pathogenicity tests on both Macis and Sugar Baby, it seemed there may be some potential for pathogenic isolates on watermelon and/or bottlegourd. After conducting a pathogenicity test for race determination on a set of susceptible watermelon cultivars, no disease was seen in all but two of the test isolates. Two isolates had 5% disease on Crimson Sweet plants which was not enough to be deemed pathogenic in a race determination test. The race 2 control of FON was confirmed as race 2 as it caused 100% disease on Sugar Baby and All Sweet as well as 95% disease on Crimson Sweet. Similarly when isolates were tested on a variety of cucurbits to determine if isolates were *Fusarium oxysporum* f. sp. *lagenariae*, no disease occurred.

In the 2013 experiment to determine the ratio of nonpathogenic to pathogenic isolates from soil dilutions in the field used for the grafting study, only one of 96 isolates was pathogenic (Table 3.10). The one isolate caused characteristic wilt symptoms in one of the six seedlings. However, there were 17 isolates that had at least one of six seeds that did not germinate. Although non-emerged seeds may have been infected by *Fusarium*, some of the seeds were not sown deeply enough in the soil and did not germinate. The race 2 isolates that served as the control caused disease in both of the

replications. When plated on Komada's medium, *F. oxysporum* was recovered from the race 2 reference isolate.

Discussion

Currently in the United States, the use of resistant cultivars is the most common way to manage Fusarium wilt. However, with increased populations of the highly aggressive race 2 of FON, the use of resistant cultivars may not give adequate disease control in years to come (Zhou & Everts, 2003; Keinath *et al.*, 2010) . With the ban on use of methyl bromide, growers are searching for more management options. Some research has shifted to focus on the use of grafting to manage Fusarium wilt (Guan *et al.*, 2012). To date, no studies exist where all three techniques were compared in one comprehensive study. In my study I examined the effects of grafting, fumigation, and a race 1 resistant cultivar on percentage area covered, disease suppression, and yield. Due to uncharacteristic symptoms of Fusarium wilt in both field seasons, isolates of *F. oxysporum* were screened through a series of pathogenicity tests to verify the causal agent of disease.

Many rootstocks used for grafting have been selected and bred for their specific disease resistance (Cohen *et al.*, 2007). In addition to having specific disease resistance qualities, rootstocks have vigorous root systems that increase the overall health of the plant (Guan *et al.*, 2012). Vigorous roots can aid in uptake of nutrients. Pathogens often

attack weak or damaged roots. With a more vigorous root system, plants will be more resistant to disease, and scion material will have increased nutrients to produce more photosynthates (Davis *et al.*, 2008). This causes the plant to look more vigorous than non-grafted plants. The Strong Tosa treatment was not as vigorous as the Emphasis treatment in either year as leaves were smaller in appearance. In the percentage area covered rating, there was a significant difference between the Strong Tosa and the Emphasis treatments in the first rating. The lower rating for Strong Tosa, which quantifies the area covered by the vines compared to the best plot, explains the observable differences in vigor seen between Strong Tosa and Emphasis treatments. Unlike in the cover crop study, different cultivars which have different growing patterns were used in the grafting study so disease severity cannot be correlated with percentage area covered ratings. By the second percentage area covered rating, there were no differences in rating. In a study evaluating rootstock characteristics, the fresh weight of plant material was higher in Emphasis-grafted plants when compared to those of Strong Tosa or the control at 25 days (Yetisir & Sari, 2003). In the next rating at 50 days after transplanting, the two grafted treatments were similar in their fresh weight plant matter. If measured in my study, a similar result would have been expected.

In 2013, Strong Tosa had the lowest disease incidence, which was significantly different from both the controls, the non-grafted and self-grafted treatments. The self-grafted treatment did not have specific disease suppression qualities like rootstocks Emphasis and Strong Tosa. This was quantified in a contrast-analyst where the disease incidence was significantly higher in the self-grafted treatment when Emphasis and

Strong Tosa treatments were grouped together (P=0.01). Disease suppression in the grafted treatments may be attributed to many factors. One such factor is the production of allelopathic chemicals. Root exudates from watermelon roots promote the germination of conidia and stimulate FON in the rhizosphere (Hao *et al.*, 2010; Ling *et al.*, 2013). In contrast, bottle gourd roots retard the growth of FON while significantly reducing conidial germination. When a watermelon scion is grafted onto a bottle gourd rootstock, the germination and growth of FON are significantly reduced compared to the non-grafted watermelon (Ling *et al.*, 2013). Chlorogenic and caffeic acids were found in the grafted and non-grafted bottle gourd exudates but not the non-grafted watermelon exudates. The production of these inhibitory chemicals could impact FON directly or stimulate microbial populations that aid in protecting the plant. Perhaps the production of these root exudates is one explanation for the disease suppression seen in grafted treatments. Disease suppression was significantly lower in both the Emphasis and Strong Tosa treatments when compared to the non-grafted control in 2013. Additionally, the two treatments also had the two highest wilt suppression index ratings. The self-graft provided no disease suppression, suggesting that it is not the grafting process that enhances disease suppression but rather the combination of rootstock and scion. The self-grafted plants likely did not produce chlorogenic and caffeic acids that could impact FON and beneficial microbes.

Another mechanism that resistant rootstocks use to achieve resistance is to form tyloses that physically block pathogens from colonizing and travelling up the vascular tissue into the susceptible scion (Kuniyasu & Takeuchi, 1983). *F. oxysporum* and FON

can effectively colonize the tissue of resistant rootstocks without causing characteristic wilting symptoms in the scion; thus resistant rootstocks serve as "asymptomatic hosts" (Malcolm *et al.*, 2013). Perhaps this is why *F. oxysporum* was isolated but caused no disease in quick or race determination pathogenicity tests in my study. These isolates were nonpathogenic in both watermelon and cucurbit race testing.

In studies in South Carolina, it has been shown that yield and number of marketable-sized fruit does not differ between Emphasis and Strong Tosa rootstocks (Keinath *et al.*, 2012; Keinath & Hassell, 2013). I found similar results in my 2012 and 2013 experiments, as no statistical differences occurred between these two rootstocks, but wilt percentage differed among grafted treatments and the self-grafted and non-grafted controls. Disease in control treatments contributed to the low yields in previous studies (Keinath *et al.*, 2012; Keinath & Hassell, 2013). In my experiment differences in disease incidence existed but did not negatively affect yield. The difference in disease incidence was smaller in my study compared to studies by Keinath and Hassell (2013) and Keinath et. al (2012). In their studies the disease incidence was 69.4 and 52.9% in the non-grafted control, while disease incidence in my non-grafted control was 22.3 and 24.7% in 2012 and 2013, respectively (back-transformed data). With higher disease pressure, yield would be negatively affected. A study in Tehran revealed that no differences in yield existed between the non-grafted control and Charleston Gray grafted onto either a *Lagenaria siceraria* or *C. maxima* X *C. moschata* rootstock (Bekhradi *et al.*, 2011) . In my study, overall yields were higher in 2012. In a very similar study, yields were almost double from one season to the next (Keinath & Hassell, 2014). The differences in yield

in my study could be due to the fact that spring was unusually cold and rainy in 2013 compared to 2012. Once the plants were transplanted in 2013, it took a few weeks for the plants to begin growing, unlike what they would do in most years. In addition, 2013 was an extremely wet growing season in which 48.5 cm of rain fell during the growing season. The rootstocks used for grafting have been selected for their vigor and ability to withstand many environmental pressures such as: temperature, flooding, drought, and salinity. For example, 'Shin-tosa'-type rootstocks have been chosen for their ability to grow under low temperatures (Davis *et al.*, 2008). Despite their ability to endure many environmental pressures, the yield suffered in the 2013 season compared to the 2012 season.

A new fumigant, dimethyl disulfide + chloropicrin, was used in the study as a comparison to the grafted treatments and the race 1 resistant cultivar. Currently, no other studies have been performed that have used dimethyl disulfide + chloropicrin as a means to control Fusarium wilt. The fumigant was statistically similar to all other treatments in percentage area covered in 2013. This suggests that the fumigant neither aided nor suppressed disease enough to cause a visual difference when compared to the non-grafted treatment in the area covered by the vines.

Disease incidence data reveals that the fumigant significantly reduced wilt in 2013 when compared to the controls, which were the non-grafted Tri-X 313 and the self-grafted Tri-X 313. Fumigants work by reducing the amount of pathogenic inoculum that can effectively colonize the roots of the watermelon plant. Significant reductions in soil CFUs were seen between the fumigated and non-fumigated control treatments. This data

was consistent with data from my cover crop study. Because lower CFUs of *F. oxysporum* were found post-fumigation than the control, less inoculum was able to infect the plants, thus less disease was found in the fumigated treatment in 2013. Only one pathogenic isolate of FON was found from soil dilutions out of 96 isolates, which suggests that the initial inoculum of FON was not very high. This may be why disease incidence was low in 2013.

In 2012, no difference in disease incidence was found between fumigated and the non-fumigated control treatment. Colony-forming units were not determined in 2012 in the grafting study. There could have been high levels of inoculum in 2012 that did not allow for a reduction in disease incidence. In 2012 during the last three rating weeks, disease incidence increased drastically in the dimethyl disulfide + chloropicrin treatment. The roots of the watermelon could have grown into areas of high CFUs of FON not affected by the fumigant, which the roots had not encountered earlier in the year. Watermelon roots have been found as far as 15 cm outside of a raised bed at the end of a growing season (Miller *et al.*, 2013). During the last few rating weeks plants were more susceptible to Fusarium wilt because they were putting on fruit and were possibly coming into contact with higher levels of FON which could have made them more susceptible. This may not have happened in 2013 because roots did not have to search for water and expand into regions that did not receive fumigant.

No differences existed between the fumigant and the control treatment in terms of yield in either 2012 or 2013. Although there were differences in disease incidence in 2013, there was not enough disease incidence or severity in the control, the non-grafted

Tri-X 313, to cause a difference in yield. Only 30% of plants were diseased in the non-grafted treatment. If more plants were severely diseased and unable to produce fruit in the control plots, then yield would have been affected.

Currently there are four races of FON. There is resistance to races 0 and 1 in commercial cultivars but no resistance to races 2 and 3. It has been demonstrated that FON populations change over time due to selection pressures. When a 4-5 year monoculture of race 1-resistant cultivars developed similar disease incidence to race-1 susceptible cultivars, it was shown that the population dynamics changed over time, not the number of CFUs of FON (Hopkins *et al.*, 1992). The longer a cultivar is used as a monoculture, the higher the proportion of race 2 isolates that are present; race 2 isolates are being selected for by using race-1 resistance. Although race-1 cultivars reduce disease, special care should be taken to avoid selection of more aggressive races over successive years when using this resistance.

'Fascination' is a race-1 resistant cultivar of seedless watermelon that I used in my two-year study. In 2013, 'Fascination' plots had 85.83% area covered by healthy vines when compared to the healthiest plot, which reflects the vigor and health of the plants in this treatment. In both years of my study, 'Fascination' treatments had 15-20% of its plants diseased in the last disease rating. This suggests that only a small percentage of FON was able to effectively colonize and cause disease in the plants. Race-1 resistant cultivars achieve resistance through a single dominant gene that prevents infection of both races 0 and 1 FON. However, the highly aggressive race 2 is still able to penetrate roots and cause a systemic infection (Wechter *et al.*, 2012). The resistance works by

reducing the amount of inoculum that can effectively cause infection, as only race 2 and 3 isolates can cause disease (Hopkins *et al.*, 1992). It has been shown that significant decreases in the colonization of stem tissue can be found when using a race-1 resistant cultivar compared to a susceptible cultivar. In a study by Zhou and Everts (2006), it was found that 33,888 FON CFUs/ g of fresh weight were found in susceptible cultivar tissues. Conversely, only 88 CFUs/ g fresh weight were found in the resistant cultivar. With considerably lower FON colonizing the tissue of the plant, the plant should experience less disease (Zhou & Everts, 2006). A similar study using susceptible Sugar Heart and moderately resistant Revolution showed that wilt percentage was significantly different between the two cultivars in two years and in two different states (Keinath *et al.*, 2010).

The yields from the 'Fascination' treatment did not differ statistically from any treatment in 2012 or 2013. In evaluations of triploid cultivars that had resistance to Fusarium wilt, 'Fascination' was one of the highest yielding cultivars when compared to other triploid cultivars (Everts & Hochmuth, 2010; Everts & Hochmuth, 2011). However, 'Fascination' had a significantly lower number of marketable-sized fruit than Emphasis and Strong Tosa rootstocks in a study by Keinath and Hassell (2013). This could be explained by the differences in disease incidence among the three treatments; 'Fascination' had 48.8% and 52.2% more disease when compared to Emphasis and Strong Tosa, respectively (Keinath & Hassell, 2013). The differences in disease incidence may be attributed to the field having confirmed populations of FON race 2.

There was a noticeable stunting in the Emphasis treatments in 2012 between rating weeks 1 and 2. The stunting found was in all replications within the Emphasis treatment. These plants remained stunted but lived throughout the field season. All of the stunted plants showed no other signs of Fusarium wilt and did not die; therefore the plants were rated as healthy in the disease ratings. Stunting can be caused by many pathogens and environmental factors. Other treatments did not have such a spike in disease from rating week 1 to rating week 2 and there were no harsh abiotic stresses. Both *Pythium* and *F. oxysporum* were isolated from diseased plant tissue in Emphasis rootstock and scion material. Isolating two pathogens from one diseased watermelon plant has been done in previous studies. In one such study, 53% of plants that had damped-off contained both *Pythium* and *Fusarium oxysporum* (Njoroge *et al.*, 2008). Normally *Pythium* prefers wet and cool conditions. Between rating weeks 1 and 2 there was only 0.03 cm of rain with an average of 24 C soil temperature. A normal irrigation schedule was followed and a sparse amount of rain fell. Emphasis and Strong Tosa rootstocks are susceptible to *Meloidogyne incognita* (root-knot nematode), which can cause stunting (Louws *et al.*, 2010). When plants were sampled for culturing no noticeable root galls, which are characteristic of root-knot nematode infection, were seen. Galling had never been observed on the roots of previous crops in the field used in 2012. Emphasis rootstock has resistance to races 1 and 2 of FON (Keinath & Hassell, 2014).

There are studies that show *Fusarium oxysporum* f. sp. *lagenariae* (FOLag) causes disease on *Lageneria* rootstocks. When rootstocks grafted with watermelon were inoculated with the pathogen, the entire plant, rootstock and scion, wilted at low

inoculum concentrations. The pathogen was found in the rootstock, but not the scion of these wilted plants. The rootstock did not show symptoms of disease until after severe wilting of the scion happened (Kuniyasu & Takeuchi, 1983). In my study, *F. oxysporum* was found in both the rootstock and scion of diseased Emphasis plants. Isolates were saved and both quick pathogenicity and race determination tests were used to determine if the isolates were pathogenic forms of FON or FOLag. None of the isolates caused disease in the watermelon race determination pathogenicity tests or in the cucurbit pathogenicity tests. It is possible that the isolates chosen were not the pathogenic forms of FON or FOLag that caused the disease in the plants; stems of diseased plants can harbor as many as 33,888 CFUs/ g (Zhou and Everts 2006).

Because no wilt was found in pathogenicity tests with saved isolates, and plants in the field did not severely wilt and die, FOLag is not a likely candidate for the disease seen in the 2012 field season. Because the plants lived, albeit stunted, but did not die, it could not be concluded what caused the stunting between rating weeks 1 and 2 in the Emphasis treatments. As grafting becomes more of an accepted practice in the United States, scientists should be prepared to face new and emerging diseases caused by selection pressures when grafting (King *et al.*, 2008).

The use of a diploid pollenizer inter-planted between triploid cultivars has become a popular practice to pollinate triploid watermelons. Pollenizers are also susceptible to diseases to which the more valued triploid watermelons are susceptible. There are many cultivars that have been specifically developed for their pollen and their disease resistance. Fusarium wilt reduces the number of staminate flowers in pollenizers

which makes them useless to a grower (Gunter & Egel, 2012). Two such cultivars used in my study as pollenizers were 'SP5' and 'SP6.' These two cultivars have resistance to races 0, 1, and 2 of FON. Many 'SP5' plants died or were severely diseased in 2012 and isolates were saved and screened. In 2013, 'SP6' pollenizers were used because they were a newer cultivar and were thought to be a hardier plant. Many of these plants also died and had very typical Fusarium wilt symptoms. In quick pathogenicity tests, none of the 'SP5' isolates were pathogenic. However, 2 of the 'SP6' isolates were FON and caused disease in both the field and in a greenhouse pathogenicity test. Either the pollenizers do not have resistance to some of the races that companies advertise or the field from where the isolates were taken from has race 3 isolates. A race determination needs to be performed to decipher these results.

When all management techniques from 2013 were grouped together in a contrast-analysis, disease was lower compared to both control treatments (P=0.002). Emphasis, Strong Tosa, or dimethyl disulfide + chloropicrin, are management tools that can significantly reduce disease. It can be concluded from the 2013 study that management tools such as grafting onto resistant rootstocks or using a fumigant can be used to provide disease suppression over the control treatments. Albeit not significantly different from the controls in both years, 'Fascination' had a consistently low disease incidence over the two-year study and should be considered as a management technique. It can also be concluded from the two-year study that Strong Tosa and Emphasis treatments performed consistently in percentage area covered and disease suppression and should be unique management tools for growers trying to manage for Fusarium wilt. Because no

differences in yield existed in either year in any treatment, choosing rootstocks for disease management should the focus of growers instead of yield.

Using a non-grafted plant with no resistance to manage Fusarium wilt may be more economically favorable for a grower; however there is risk in doing so. Commercial watermelon fields have been known to collapse, sometimes reaching upwards of 50% of plants exhibiting symptoms of Fusarium wilt (Paret, 2013). Because grafted transplants cost up to three times as much as a non-grafted plant, many growers are hesitant to use grafted plants because of the cost (Keinath & Hassell, 2014). When the average of the marketable yields from both 2012 and 2013 were used to calculate a grower's net return, it was not economically favorable to use grafted plants. Emphasis plants would return a grower $37/ha while Strong Tosa would give a grower a negative return of $2,313/ha (Ferreira, 2013). Using the non-grafted Tri-X 313 would return a grower the most profit, $1,370/ha (Welker, 2013). Although in my study no differences were seen in yield, Keinath and Hassell (2014) showed the potential economic benefits of using the more costly grafted plant. Even in years akin to my study where no differences existed in yield, the risk of losing half of the plants to Fusarium wilt because a cultivar with no resistance or a non-grafted plant were chosen should be avoided.

Literature Cited

Bekhradi F, Kashi A, Deishad M, 2011. Effect of Three Cucurbits Rootstocks on Vegetative and Yield of 'Charleston Gray' Watermelon. *International Journal of Plant Production* 5, 105-109.

Boughalleb N, Tarchoun N, Mbarki EA, and Mahjoub EA, 2007. Resistance Evaluation of Nine Cucurbit Rootstocks and Grafted Watermelon (*Citrullus lanatus* L.) Varieties Against *Fusarium* Wilt and *Fusarium* Crown and Root Rot. *Journal of Plant Sciences* 2, 102-107.

Cohen R, Burger Y, Horev C, Koren A, Edelstein M, 2007. Introducing Grafted Cucurbits to Modern Agriculture - The Israeli Experience. *Plant Disease* 91, 916-923.

Davis AR, Perkins-Veazie P, Sakata Y, Lopez-Galarza S, Marota JV, Lee S, Huh Y, Sun Z, Miguel A, King SR, Cohen R, and Lee J, 2008. Cucurbit Grafting. *Critical Reviews in Plant Sciences* 27, 50-74.

Everts KL, Hochmuth M, 2010. Field Evaluation of Triploid Cultivars for Resistance to Fusarium Wilt of Watermelon in Delaware, 2010. *Plant Disease Management Reports* 5:V175.

Everts KL, Hochmuth M, 2011. Evaluation of Triploid Cultivars for Resistance to Fusarium Wilt of Watermelon, 2011. *Plant Disease Management Reports* 6:V014.

Ferreira, W. 2013. Enterprise Budges: Watermelons - On plastic - Drip irrigation-Seedless. Online. Clemson Cooperative Extension.

Guan W, Zhao X, Hassell R, Thies J, 2012. Defense Mechanisms Involved in Disease Resistance of Grafted Vegetables. *HortScience* 47, 164-170.

Gunter C, Egel DS, 2012. Staminate Flower Production and Fusarium Wilt Reaction of Diploid Cultivars Used as Pollenizers for Triploid Watermelon. *HortTechnology: Preliminary and Regional Reports* 22, 694-699.

Hao W, Ren L, Ran W, Shen Q, 2010. Allelopathic Effects of Root Exudates from Watermelon and Rice Plants on Fusarium oxysporum f.sp. niveum. *Plant Soil* 336, 485-497.

Hopkins DL, Lobinske RJ, Larkin RP, 1992. Selection for Fusarium-Oxysporum F Sp-Niveum Race-2 in Monocultures of Watermelon Cultivars Resistant to Fusarium-Wilt. *Phytopathology* 82, 290-293.

Keinath AP, Hassell RL, 2013. On-farm Evaluation of Grafted Seedless Watermelon for Control of Fusarium Wilt Race 2, 2012. *Plant Disease Management Reports* 7:V033.

Keinath AP, Hassell RL, 2014. Control of Fusarium Wilt of Watermelon by Grafting onto Bottlegourd or Interspecific Hybrid Squash Despite Colonization of the Rootstocks by Fusarium oxysporum. *Plant Disease "First Look"*.

Keinath AP, Hassell RL, DuBose VB, 2012. Field Evaluation of Six Cucurbit Rootstocks to Manage Fusarium Wilt on Triploid Watermelon, 2011. *Plant Disease Management Reports* 6:V024.

Keinath AP, Hassell RL, Everts KL, Zhou X, 2010. Cover Crops of Hybrid Common Vetch Reduce Fusarium Wilt of Seedless Watermelon in the Eastern United States. *Plant Health Progress* doi:10.1094/PHP-2010-0914-01-RS.

King SR, Davis AR, Wenge L, Levi A, 2008. Grafting for Disease Resistance. *HortScience* 43, 1673-1676.

Kleczewski NM, Egel DS, 2011. A Diagnostic Guide for Fusarium Wilt of Watermelon. *Plant Health Progress* doi:10.1094/PHP-2011-1129-01-DG.

Kubota C, McClure MA, Kokalis-Burelle N, Bausher MG, Rosskopf EN, 2008. Vegetable Grafting: History, Use, and Current Technology Status in North America. *HortScience* 43, 1664-1669.

Kuniyasu K, Takeuchi S, 1983. Wilt of Watermelon Grafted on Bottle Gourd Rootstocks Inoculated with Fusarium-Oxysporum-F-Sp-Lagenariae. *Bulletin of the Vegetable and Ornamental Crops Research Station Series A*, 139-140.

Ling N, Zhang W, Wang D, Mao J, Huang Q, Guo S, Shen Q, 2013. Root Exudates from Grafted-Root Watermelon Showed a Certain Contribution in Inhibiting Fusarium oxysporum f. sp niveum. *Plos One* 8, e63383.

Louws FJ, Rivarda CL, Kubotac C, 2010. Grafting Fruiting Vegetables to Manage Soilborne Pathogens, Foliar Pathogens,Arthropods and Weeds. *Scientia Horticulturae* 127, 127-146.

Malcolm GM, Kuldau GA, Gugino BK, Jimenez-Gasco MM, 2013. Hidden Host Plant Associations of Soilborne Pathogens: An Ecological Perspective. *Phytopathology* 103, 538-544.

Miller G, Khalilian A, Adelberg JW, Farahani HJ, Hassell RL, Wells CW, 2013. Grafted Watermelon Root Length Density and Distribution under Different Soil Moisture Treatments. *HortScience* 48, 1021-1026.

Namiki F, Shiomi T, Kayamura T, Tsuge T, 1994. Characterization of the Formae Speciales of Fusarium-Oxysporum Causing Wilts of Cucurbits by Dna-Fingerprinting with Nuclear Repetitive Dna-Sequences. *Applied and Environmental Microbiology* 60, 2684-2691.

Njoroge SM, Riley MB, Keinath AP, 2008. Effect of Incorporation of *Brassica* spp. Residues on Population Densities of Soilborne Microorganisms and on Damping-off and Fusarium Wilt of Watermelon. *Plant Disease* 92:2, 287-294.

Paret ML, 2013. *Personal Communication.*

Robinson RW, Decker-Walters DS, 1997. *Cucurbits.* New York, NY: CAB International.

Wechter WP, Kousik C, McMillan M, Levi A, 2012. Identification of Resistance to Fusarium oxysporum f. sp niveum Race 2 in Citrullus lanatus var. citroides Plant Introductions. *HortScience* 47, 334-338.

Welker R, 2013. *Personal Communication.*

Yetisir H, Sari N, 2003. Effect of Different Rootstock on Plant Growth, Yield and Quality of Watermelon. *Australian Journal of Experimental Agriculture* 43, 1269-1274.

Yetisir H, Sari N, Yucel S, 2003. Rootstock Resistance to Fusarium Wilt and Effect on Watermelon Fruit Yield and Quality. *Phytoparasitica* 31, 163-169.

Zhou XG, Everts KL, 2006. Suppression of Fusarium Wilt of Watermelon Enhanced by Hairy Vetch Green Manure and Partial Cultivar Resistance. *Plant Health Progress* doi:10.1094/PHP-2006-0405-01-RS.

Zhou XG, Everts KL, 2003. Races and Inoculum Density of Fusarium oxysporum f. sp niveum in Commercial Watermelon Fields in Maryland and Delaware. *Plant Disease* 87, 692-698.

Zhou XG, Everts KL, 2004. Suppression of Fusarium Wilt of Watermelon by Soil Amendment with Hairy Vetch. *Plant Disease* 88, 1357-1365.

Table 3.1 Colony-formining units (CFU) of *Fusarium oxysporum* in fumigated and non-fumigated soil sampled in April and May, 2013

Scion	**Fumigant**	**April *Fusarium oxysporum* CFU X 10^{-2}/10g soil at 0-10 cm depth**	**April *Fusarium oxysporum* CFU X 10^{-2}/10g soil at 10-20 cm depth**	**April *Fusarium oxysporum* combined depths**	**May *Fusarium oxysporum* CFU X 10^{-2}/10g soil at 0-10 cm depth**	**May *Fusarium oxysporum* CFU X 10^{-2}/10g soil at10- 20 cm depth**	**May *Fusarium oxysporum* combined depths**
Tri-X 313	**Dimethyl disulfide + chloropicrin[x]**	4.58 a[y]	11.03 a	7.80 a A	5.91 a	5.32 a	5.61 a A
Tri-X 313	**None**	54.32 b	50.41b	52.36 b A	32.61 b	22.84 b	27.72 b B

[x] A 79:21 rate of dimethyl disulfide + chloropicrin was used for fumigation.

[y] Means within the same column followed by the same lower case letter are not significantly different and means within the same row followed by an upper case letter are not significantly different, Waller-Duncan k-ratio *t* test, k=100 or *P*=0.05.

Table 3.2 Week 2-9 Percentage of diseased plants based on disease incidence, area under the disease progress curve, and wilt suppression index, 2012

Treatment									
Scion	**Rootstock**	**Fumigant**	**Week 1**	**Week 3**	**Week 5**	**Week 7**	**Week 9**	**AUDPC[w]**	**Wilt Suppression Index[x]**
Tri-X 313	**Emphasis**	**None**	0.24 a[y]	0.95 a	4.71 a	8.11 a	12.34 a	357 a	49.49
Tri-X 313	**Strong Tosa**	**None**	0.24 a	2.13 a	3.76 a	5.36 a	8.04 a	325 a	50.00
Fascination	**Non grafted**	**None**	0.00 a	4.53 a	4.90 a	5.91 a	10.26 a	587 a	21.72
Tri-X 313	**Tri-X 313**	**None**	0.00 a	2.27 a	2.72 a	6.11 a	15.08 a	537 a	11.11
Tri-X 313	**Non grafted**	**Dimethyl disulfide + chloropicrin[z]**	0.00 a	5.36 a	8.01 a	15.93 a	28.17 a	770 a	0.00
Tri-X 313	**Non grafted**	**None**	0.95 a	7.99 a	10.20 a	11.26 a	22.34 a	694 a	0.00

[w] Area Under the Disease Progress Curve.

[x] Wilt Suppression Index (WSI), with the scale of 0 (no suppression) to 100% (total suppression), was calculated by WSI = $(X_{nv}-X_v)/X_{nv}*100$, in which X_{nv} = mean percent wilt in the non-fumigated, non-grafted control, and X_v = mean percent wilt in the that treatment.

[y] All rating data were transformed using arc-sine square root analyzed, and then back-transformed as shown. Means within the same column followed by the same letter are not significantly different, Waller-Duncan k-ratio *t* test, k=100 or *P*=0.05.

[z] A 79:21 rate of dimethyl disulfide + chloropicrin was used for fumigation.

Table 3.3 Week 2-9 Percentage of diseased plants based on disease incidence, area under the disease progress curve, and wilt suppression index, 2013

Treatment									
Scion	**Rootstock**	**Fumigant**	**Week 2**	**Week 4**	**Week 6**	**Week 8**	**Week 9**	**AUDPC**	**Wilt Suppression Index[x]**
Tri-X 313	**Emphasis**	**None**	0.00 b[y]	0.00 a	0.25 a	0.25 b	2.23 cd	44 a[y]	71.58
Tri-X 313	**Strong Tosa**	**None**	0.00 b	1.00 a	1.00 a	1.00 b	1.00 d	129 a	80.91
Fascination	**Non grafted**	**None**	0.00 b	1.00 a	1.00 a	4.76 ab	10.50 bc	222 a	36.93
Tri-X 313	**Tri-X 313**	**None**	1.00 a	2.23 a	3.57 a	13.76 a	22.98 ab	418 a	4.09
Tri-X 313	**Non grafted**	**Dimethyl disulfide + chloropicrin[z]**	0.00 b	2.86 a	2.86 a	2.86 b	8.18 cd	260 a	45.22
Tri-X 313	**Non grafted**	**None**	0.25 ab	1.95 a	7.64 a	12.41 a	24.69 a	553 a	0.00

[w] Area Under the Disease Progress Curve.

[x] Wilt Suppression Index (WSI) , with the scale of 0 (no suppression) to 100% (total suppression), was calculated by WSI = $(X_{nv}-X_v)/X_{nv}*100$, in which X_{nv} = mean percent wilt in the non-fumigated, non-grafted control, and X_v = mean percent wilt in that treatment.

[y] All rating data were transformed using arc-sine square root analyzed, and then back-transformed as shown. Rating weeks 8 and 9 were analyzed using repeated measures which compared treatments within each rating week. Means within the same column followed by the same letter are not significantly different, Waller-Duncan k-ratio *t* test, k=100 or *P*=0.05.

[z] A 79:21 rate of dimethyl disulfide + chloropicrin was used for fumigation.

Figure 3.1Disease progress curve from grafting study, 2012

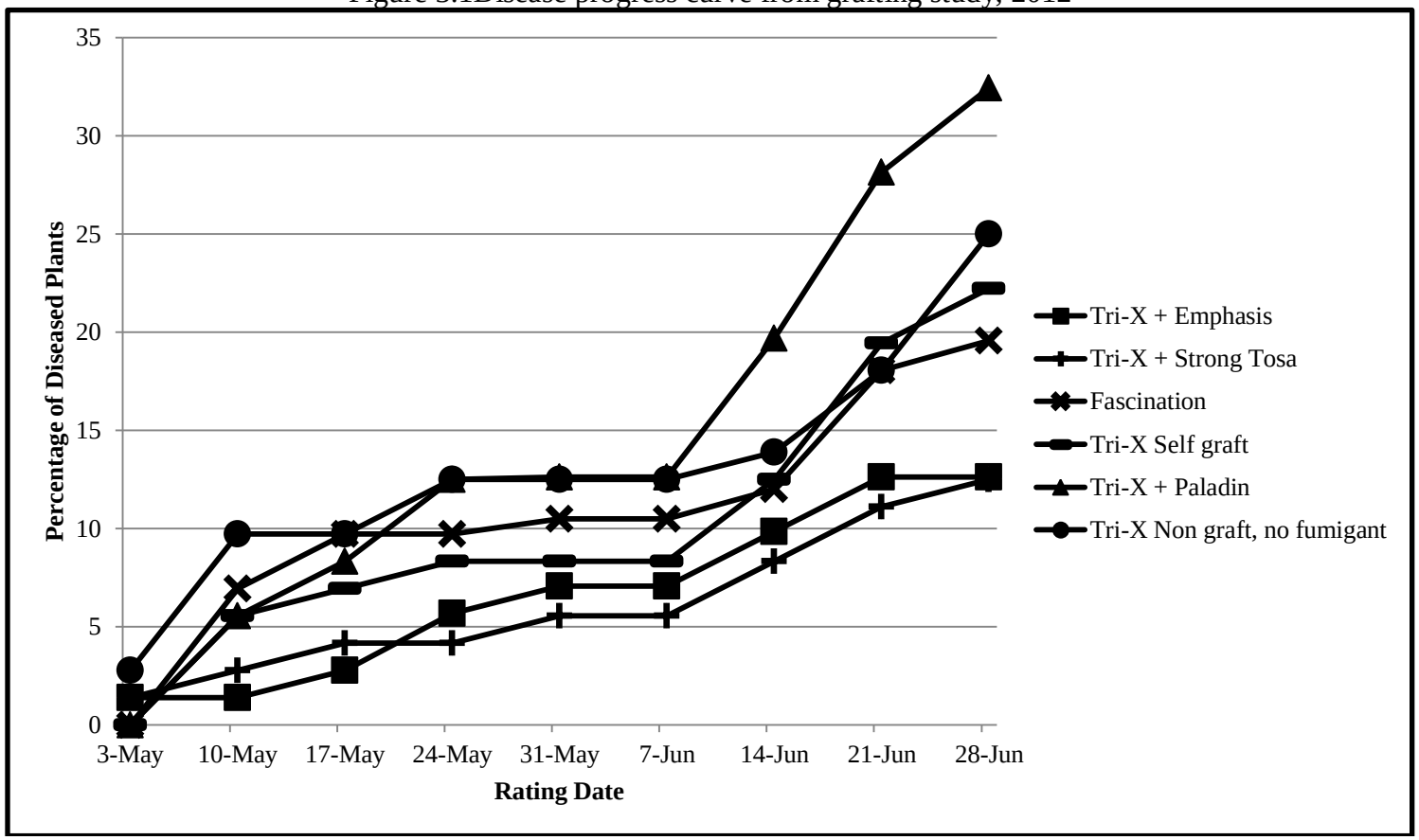

Figure 3.2 Disease progress curve from grafting study 2013

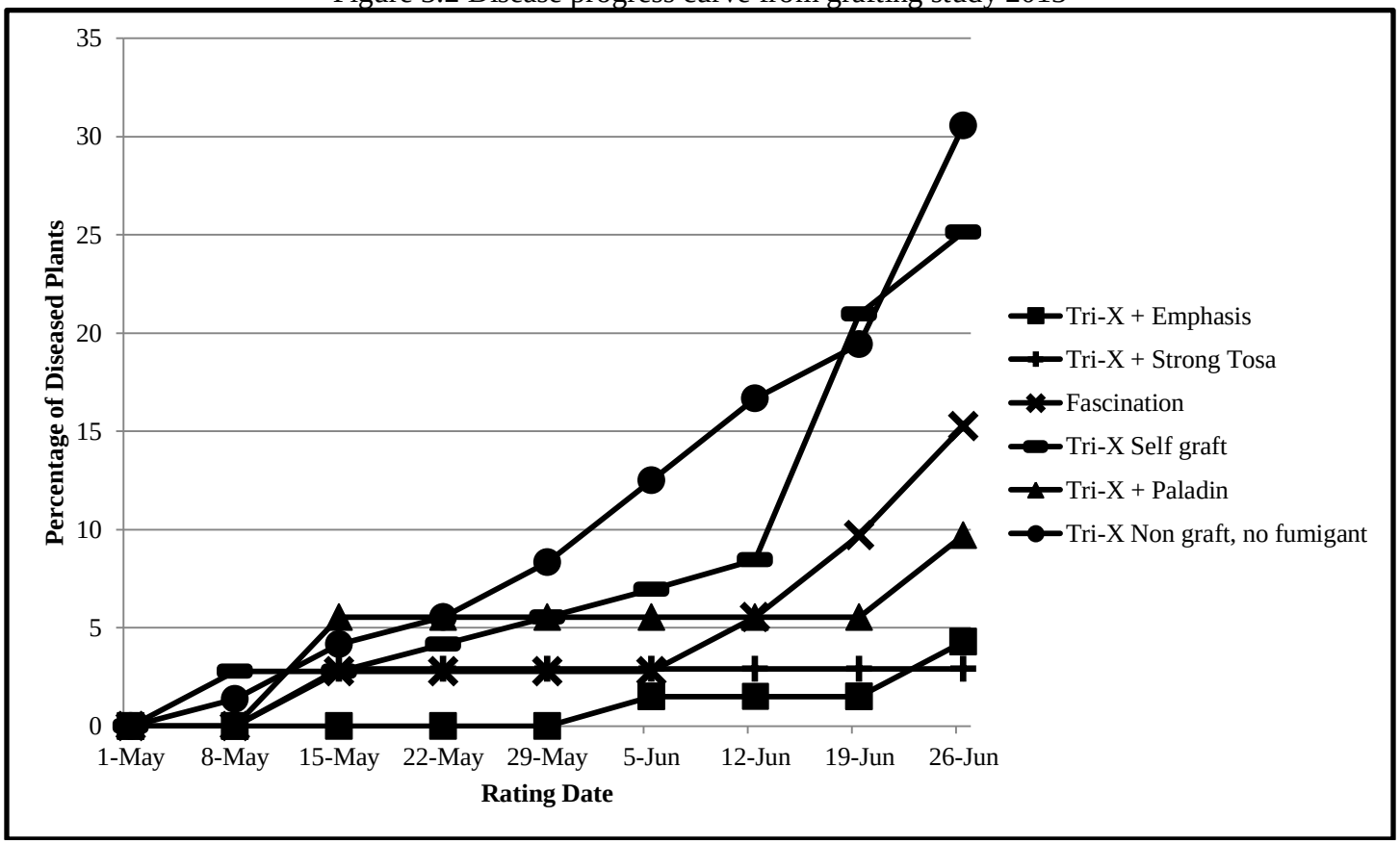

Table 3.4 Percentage area covered in grafting study, 2013

Treatment					
Scion	**Rootstock**	**Fumigant**	**Percentage Area Covered**[x]	**Percentage Area Covered**	**Change in Percentage Area Covered**
Tri-X 313	**Emphasis**	**None**	84.17 a[y]	73.33 a	-10.84
Tri-X 313	**Strong Tosa**	**None**	58.33 b	68.33 a	10.00
Fascination	**Non grafted**	**None**	75.83 ab	85.83 a	10.00
Tri-X 313	**Tri-X 313**	**None**	84.17 a	72.50 a	11.67
Tri-X 313	**Non grafted**	**Dimethyl disulfide+chloropicrin**[z]	75.00 ab	81.67 a	6.67
Tri-X 313	**Non grafted**	**None**	75.83 ab	75.83 a	0.00

[x] Percentage area covered ratings compared the percentage area covered by vines in individual plots to the percentage area covered in the plot with the healthiest and most vigorous vines. Ratings were taken on 30 May and 19 Jun 2013.

[y] Means within the same column followed by the same letter are not significantly different, Waller-Duncan k-ratio *t* test, k=100 or *P*=0.05.

[z] A 79:21 rate of dimethyl disulfide + chloropicrin was used for fumigation.

Table 3.5 Average marketable yield and average number of marketable watermelons, 2012

Treatment					
Scion	**Rootstock**	**Fumigant**	**Average Total Marketable Yield (kg/15.24m)**	**Average Total Marketable Yield (kg/ha)**	**Average Number of Marketable Watermelons per Treatment**
Tri-X 313	**Emphasis**	**None**	232.95 a	41763 a	33.50a
Tri-X 313	**Strong Tosa**	**None**	178.27 a	31960 a	24.17a
Fascination	**Non grafted**	**None**	207.51 a	37203 a	29.33a
Tri-X 313	**Tri-X 313**	**None**	239.26 a	42895 a	33.50a
Tri-X 313	**Non grafted**	**Dimethyl disulfide + chloropicrin**	214.67 a	38486 a	29.83a
Tri-X 313	**Non grafted**	**None**	215.41 a	38619 a	31.17a

[x] A 79:21 rate of dimethyl disulfide + chloropicrin was used for fumigation.

[y] Means within the same column followed by the same letter are not significantly different, Waller-Duncan k-ratio *t* test, k=100 or *P*=0.05.

Table 3.6 Average marketable yield and average number of marketable watermelons, 2013

Treatment					
Scion	**Rootstock**	**Fumigant**	**Average Marketable Yield (kg/14.3m)**	**Average Marketable Yield (kg/ha)**	**Average Number of Marketable Watermelons per Treatment**
Tri-X 313	**Emphasis**	**None**	162.04 a[y]	30961 a	20.50 a
Tri-X 313	**Strong Tosa**	**None**	122.21 a	23350 a	21.50 a
Fascination	**Non grafted**	**None**	150.23 a	28705 a	23.67 a
Tri-X 313	**Tri-X 313**	**None**	140.73 a	26888 a	26.00 a
Tri-X 313	**Non grafted**	**Dimethyl disulfide+chloropicrin[z]**	167.24 a	31954 a	26.67 a
Tri-X 313	**Non grafted**	**None**	149.81 a	28623 a	20.17 a

[y] Means within the same column followed by the same letter are not significantly different, Waller-Duncan k-ratio *t* test, k=100 or *P*=0.05.

[z] A 79:21 rate of dimethyl disulfide + chloropicrin was used for fumigation.

Table 3.7 *Fusarium oxysporum* and *Pythium* isolation data from diseased plants in grafting study, 2012

	Treatment Sampled			**Roots**		**Stem/Vines**[u]	**Rootstock**	**Scion**
Date Sampled	**Scion**	**Rootstock**	**Fumigant**	***Fusarium oxysporum***[v]	***Pythium***	***Fusarium oxysporum***	***Fusarium oxysporum***	
5/25/12	**Tri-X 313**	**Emphasis**	**None**	8/20[w]	4/20	--[x]	8/10	5/10
6/12/12	**Tri-X 313**	**Emphasis**	**None**	4/20	16/20	--	10/10	7/10
6/22/12	**Tri-X 313**	**Non grafted**	**None**	--	--	6/8	--	--
7/2/12	**Tri-X 313**	**Emphasis**	**None**	--	--	24/24	--	--
7/23/12[y]	**Tri-X 313**	**Emphasis**	**None**	--	0/8	8/8	--	--
	Tri-X 313	**Strong Tosa**	**None**	--	0/8	6/8	--	--
	Fascination	**Non grafted**	**None**	--	3/16	12/16	--	--
	Tri-X 313	**Non grafted**	**Dimethyl disulfide + chloropicrin**[z]	--	1/8	7/8	--	--
	Tri-X 313	**Non grafted**	**None**	--	5/8	6/8	--	--

[u] Samples were taken from both the stem and vine of plants; two pieces from the stem (below the crown) and two pieces from the vine of each plant sample were plated. No distinction was made between rootstock and scion of grafted plants.
[v] Plants sampled showed non-typical Fusarium wilt symptoms.
[w] Each row refers to plants from that treatment; numbers correspond to the number of pieces with the pathogen present; in most cases four pieces from each plant were cultured.
[x] -- means that no samples were cultured.
[y] One healthy and one diseased plant from one replication in each treatment. One healthy and one diseased plant were chosen from two plots in the 'Fascination' treatment, for a total of four plants sampled.
[z] A 79:21 rate of dimethyl disulfide + chloropicrin was used for fumigation.

Table 3.8 *Fusarium oxysporum* isolation data from one healthy and one diseased plant in each plot post-harvest, 2012

Treatment			Healthy Plant		Diseased Plant	
Scion	**Rootstock**	**Fumigant**	**Rootstock**	**Scion**	**Rootstock**	**Scion**
Tri-X 313	**Emphasis**	**None**	8/12[x]	11/12	6/12	10/12
Tri-X 313	**Strong Tosa**	**None**	8/12	9/12	9/12	9/12
Fascination	**Non grafted**	**None**	--[y]	16/24	--	21/24
Tri-X 313	**Tri-X 313**	**None**	10/12	10/12	10/12	11/12
Tri-X 313	**Non grafted**	**Dimethyl-disulfide + chloropicrin[z]**	--	21/24	--	16/24
Tri-X 313	**Non grafted**	**None**	--	21/24	--	16/24

[x] Each row refers to plants from that treatment; numbers correspond to the number of pieces with the pathogen present; in all cases two pieces from each plant were taken

[y] -- means that either the roots or stem were sampled, but not both.

[z] A 79:21 rate of Paladin + chloropicrin was used for fumigation.

Table 3.9 *Fusarium oxysporum* and *Pythium* isolation data from diseased plants in grafting study 2013

	Treatment			**Roots**		**Stem/Vines**[u]	**Rootstock**	**Scion**
Date Sampled	**Scion**	**Rootstock**	**Fumigant**	***Fusarium oxysporum***[w]	***Pythium***	***Fusarium oxysporum***	***Fusarium oxysporum***	
5/1/13	**Tri-X 313**	**Tri-X 313**	**None**	--[x,y]	4/4	0/4	--	--
5/8/13	**Tri-X 313**	**Tri-X 313**	**None**	0/8	7/7	--	6/8	2/8
5/15/13	**Tri-X 313**	**Strong Tosa**	**None**	0/4	4/4	--	1/2	0/2
	Tri-X 313	**Non Grafted**	**None**	3/3	1/3	--	--	--
	Tri-X 313	**Non grafted**	**Dimethyl disulfide + chloropicrin**[z]	0/4	3/4	--	--	--
5/22/13	**Tri-X 313**	**Strong Tosa**	**None**	4/4	0/4	1/2	--	--
	Tri-X 313	**Non grafted**	**None**	0/4	2/4	2/4	--	--
	Tri-X 313	**Non grafted**	**Dimethyl disulfide + chloropicrin**	--	--	4/4	--	--

[u] Samples were taken from both the stem and vine of plants; two pieces from the stem (below the crown) and two pieces from the vine of each plant sample were plated. No distinction was made between rootstock and scion of grafted plants.
[w] Plants sampled showed non-typical Fusarium wilt symptoms.
[x] -- means that no samples were cultured.
[y] Each row refers to one or two plants; numbers correspond to the number of pieces with the pathogen present; in most cases four pieces from each plant were taken.
[z] A 79:21 rate of dimethyl disulfide + chloropicrin was used for fumigation.

Table 3.10 Pathogenicity testing on Emphasis, 'SP5,' and 'SP6' isolates collected from field studies 2012-2013.

Pathogenicity Test	**Cultivar Used**	**Source of Isolates Tested**	**Number of Pathogenic Isolates**
Quick Pathogenicity 1	Sugar Baby (*Citrullus lanatus*)	Emphasis rootstock and Tri-X 313 scion	0/8[y]
Quick Pathogenicity 1	Sugar Baby (*Citrullus lanatus*)	'SP5' pollenizers	0/5
Quick Pathogenicity 2	Macis (*Lagenaria siceraria*)	Emphasis rootstock and Tri-X 313 scion	0/8
Quick Pathogenicity 2	Macis (*Lagenaria siceraria*)	'SP5' pollenizers	0/5
Full Pathogenicity 3	Differential Watermelon Cultivars	Emphasis rootstock and Tri-X 313 scion	0/8
Full Pathogenicity 3	Differential Watermelon Cultivars	'SP5' pollenizers	0/5
Full Pathogenicity 4	Differential Watermelon Cultivars	Emphasis rootstock and Tri-X 313 scion	0/8
Full Pathogenicity 5	Differential Cucurbit Cultivars	Emphasis rootstock and Tri-X 313 scion	0/8
Quick Pathogenicity 6	Black Diamond (*Citrullus lanatus*)	'SP6' pollenizers	2/24
Quick Pathogenicity 7	Black Diamond (*Citrullus lanatus*)	Soil Dilution Isolates	1/96

[y] The first number represents the number of pathogenic isolates and the second number represents the total number of isolates tested.

CHAPTER FOUR

Conclusion

Fusarium wilt has become the biggest threat to watermelon growers in South Carolina in recent years. The absence of methyl bromide, lack of available land for crop rotation, the lack of race 2 and 3 resistance, the need for resistant cultivars, and increased acreage of susceptible seedless watermelon cultivars, has increased the need for more Fusarium wilt management tools. The goal of this two-year study was to evaluate alternative management tools that growers could use as part of an integrated approach to manage Fusarium wilt.

Cover cropping with hairy vetch and incorporation into the soil before watermelon is grown has been shown to reduce Fusarium wilt. Explanations as to why suppression is achieved in Maryland but not South Carolina may be due to many sources of variability among field sites. Sources of variability can include but are not limited to: soil organic matter, soil type, pathogen populations, beneficial populations of soil microbes, cultural practices, and weather conditions. Under the experimental conditions, the hairy vetch cover crop significantly increased population levels of *F. oxysporum* in the soil while the fumigants, dimethyl disulfide + chloropicrin and methyl bromide + chloropicrin, significantly reduced *F. oxysporum*. Watermelon yields were not significantly different in the cover crop treatment compared with other treatments. These findings are consistent with other cover crop studies (Zhou & Everts, 2004; Zhou & Everts, 2006). In one out of three experiments, dimethyl disulfide + chloropicrin reduced both CFUs of *F. oxysporum* and disease incidence. More studies need to be done to

analyze the effects of dimethyl disulfide + chloropicrin on Fusarium wilt before it can be considered a viable disease management technique.

The grafting study was the first study to examine the effects of grafting, a race-1 resistant cultivar, and fumigation on Fusarium wilt in one comprehensive study. Watermelon grafted onto the rootstocks used, Emphasis (*Lagenaria siceraria*) and Strong Tosa (*C. maxima* X *C. moschata*), consistently had a lower disease incidence in the two-year study than the other treatments. In other studies evaluating disease incidence of Fusarium wilt, both Emphasis and Strong Tosa significantly lowered disease when compared to the non-grafted controls (Keinath & Hassell, 2013; Keinath & Hassell, 2014). Although the grafted treatments had a lower disease incidence in my study, there were no significant differences in yield among treatments in either year. Although the cost of using grafted transplants is three times as high as using non-grafted transplants, growers should be aware of the risks involved with using non-grafted transplants that are susceptible to Fusarium wilt (Davis *et al.*, 2008). In commercial fields, Fusarium wilt has been found in over 50% of the plants (Keinath & Hassell, 2008). This could result in a substantial yield loss that could have been prevented with the use of grafted transplants. Currently, use of grafted plants is the best strategy to manage Fusarium wilt in watermelon production.

The race 1-resistant cultivar, 'Fascination', consistently suppressed disease in this two-year study. It has been shown that monocropping race 1-resistant cultivars can select for the more virulent race 2 and 3 isolates (Hopkins *et al.*, 1992; Zhou *et al.* 2010). Using race 1-resistance is an excellent management tool for Fusarium wilt; however,

precautions must be taken to ensure that selection of more virulent races does not occur by successive plantings of race 1-resistant cultivars.

In an effort to manage Fusarium wilt, an integrated approach must be taken. Many techniques must be employed and alternated from year to year. Alternation of disease management techniques leads to a more sustainable watermelon production system over time. The current, most promising techniques for management of Fusarium wilt are the use of grafted plants in conjunction with race 1-resistant cultivars. While these two techniques can be alternated into a management system, a grower must know a field's previous disease history before using race1-resistant cultivars. For example, if a field has had a previous history of Fusarium wilt despite deployment of race 1-resistance, then use of disease resistant cultivars would not be effective. Instead they may choose to rotate resistant rootstocks from year to year or fumigate every few years to reduce the pathogenic inoculum. Although managing Fusarium wilt is necessary for watermelon growers, a main concern for a grower is producing a profitable crop. Considerations and risks must be analyzed and taken every year by each grower on an individual farm basis.

Literature Cited

Davis AR, Perkins-Veazie P, Sakata Y, Lopez-Galarza S, Marota JV, Lee S, Huh Y, Sun Z, Miguel A, King SR, Cohen R, and Lee J, 2008. Cucurbit Grafting. *Critical Reviews in Plant Sciences* 27, 50-74.

Hopkins DL, Lobinske RJ, Larkin RP, 1992. Selection for Fusarium-Oxysporum F Sp-Niveum Race-2 in Monocultures of Watermelon Cultivars Resistant to Fusarium-Wilt. *Phytopathology* 82, 290-3.

Keinath AP, Hassell RL, 2013. On-farm Evaluation of Grafted Seedless Watermelon for Control of Fusarium Wilt Race 2, 2012. *Plant Disease Management Reports* 7:V033.

Keinath AP, Hassell RL, 2014. Control of Fusarium Wilt of Watermelon by Grafting onto Bottlegourd or Interspecific Hybrid Squash Despite Colonization of the Rootstocks by Fusarium oxysporum. *Plant Disease.* doi/pdf/10.1094/PDIS-01-13-0100-RE

Keinath AP, Hassell RL, 2008. On-farm Evaluation of Hairy Vetch and Fumigation for Integrated Control of Fusarium Wilt on Seedless Watermelon, 2008. *Plant Disease Management Reports* 3:V035.

Zhou XG, Everts KL, 2006. Suppression of Fusarium Wilt of Watermelon Enhanced by Hairy Vetch Green Manure and Partial Cultivar Resistance. *Plant Health Progress* doi:10.1094/PHP-2006-0405-01-RS.

Zhou XG, Everts KL, 2004. Suppression of Fusarium Wilt of Watermelon by Soil Amendment with Hairy Vetch. *Plant Disease* 88, 1357-1365.

Zhou XG, Everts KL, Bruton BD, 2010. Race 3, a New and Highly Virulent Race of Fusarium oxysporum f. sp niveum Causing Fusarium Wilt in Watermelon. *Plant Disease* 94, 92-98.